Case Study: Module 3 Models in Neurobiology (Part 2)

Methods in Neurobiology

Overview

In this assignment you will be paired with another student and ask to analyze the model that he/she set up in Part 1 (Module 2).
This a 20-point assignment.

Instructions

Once paired with another student, you will have to review your coworker's project illustrated in Part 1. The review will have to include:

1. An initial sentence/small paragraph to summarize the project you are reviewing

Then answer the following questions using at least 1-2 sentences/ bullet points:

- Do you think that their model is scientifically sound and appropriate? Why?
- Is there another model as good as the one presented that could be used to obtain the same degree of information (for example a model that requires less invasive procedure)? Why?
- Is there another model that could be used to have more detailed and extensive information?

2. References can be used. Your work should be 1 page max (not including citations).

Few examples follow.

Example One

Question/Biological Problem (10 points)
Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset neurodegenerative disorder characterized by the progressive loss of motor neurons in the brain, brainstem, and spinal cord, which culminates in paralysis and death within a few years of diagnosis[1]. New technologies for gene mapping have enabled the identification of nearly 30 genes in ALS pathogenesis. The first gene discovered associated with familiar forms of ALS is SOD1[2]. To date, over 180 different mutations have been described in SOD1 gene whose function is still unknown. One of the earliest and most common mutations discovered is a glycine to alanine substitution in position 93 of the protein.

AIM: To build a model to mimic how the mutant form of SOD1, SOD1 G93A, can cause cellular death and impact normal function of the endogenous, wild-type form of SOD1.

Research Model and Plan (10 points)

- Through transgenesis, a DNA vector carrying a mutant copy of human SOD1 G93A gene will be inserted stably into human neuroblastoma SH-SY5Y cells, an immortalized cell line commonly used to study brain disorders. After transgenesis, cells will be selected using appropriate selection markers (such as gene products to antibiotics resistance). Within the same DNA vector, the SOD1 mutant will be tagged at the C-terminal with a fluorescent protein such as GFP to facilitate identification of cell clones in live cell imaging. Thus, this line will become a stable cell line for mutant SOD1 expression and can be used to study how mutant SOD1 impacts the function of the endogenous wild-type form of the gene.
- This cell line is the most appropriate model compared to primary cell cultures because immortalized cell lines can be cultured indefinitely and do not require repeated human or animal sampling.

- Compared to brain slices that require more advanced/complicated techniques, it is easier to express exogenous genes in cell lines.

1) Beckman JS, Estévez AG, Crow JP, Barbeito L. Superoxide dismutase and the death of motoneurons in ALS. Trends Neurosci. 2001 Nov;24(11 Suppl):S15-20. doi: 10.1016/s0166-2236(00)01981-0.
2) Sau D, De Biasi S, Vitellaro-Zuccarello L, Riso P, Guarnieri S, Porrini M, Simeoni S, Crippa V, Onesto E, Palazzolo I, Rusmini P, Bolzoni E, Bendotti C, Poletti A. Mutation of SOD1 in ALS: a gain of a loss of function. *Human Molecular Genetics*, Volume 16, Issue 13, 1 July 2007, Pages 1604–1618. doi:10.1093/hmg/ddm110

Part 2:

- In this project, Ms. A, focus on studying the function of mutant SOD1 using human neuroblastoma cell line, a cell model that aims to mimic brain cells in vitro. The SOD1 G93A gene will be expressed as a stable transgene in these cells.
- The use of cell lines to study gene function, in particular in this case, human neuroblastoma lines, for the expression of SOD 1, is a simple but appropriate model that can recapitulate some of the features of CNS disease in a dish. Such model can be useful to study the impact of mutations on SOD1 function and its direct consequences on cell functionality.
  A similar approach to the one described above could be to develop a transgenic yeast model. Yeast is the simplest and one of the most studied eukaryotic organisms. In addition, most of the cellular mechanisms present in higher order eukaryotes are well conserved in yeast. Thus, it is plausible that expression of mutant SOD 1 in yeast results in metabolic alterations similar to what happens in mammalian cells.
- Another appropriate model would be to develop a transgenic animal model such as a mouse or *C. Elegans*, a more comprehensive system to study complex disease. This model could provide insights in how an increased expression of SOD 1 affects directly motoneuron signaling.

Example Two

Question/Biological Problem (10 points)

Recently, the efficacy of antidepressant agents has been a matter of controversy. The selective serotonin reuptake inhibitors (SSRIs), such as citalopram and fluoxetine (Prozac), are currently considered to be a first-line therapeutic tool for the treatment of depression, even though less than 40% of treated patients respond to this type of antidepressant[1]. SSRIs are thought to moderate depressive symptoms by mainly enhancing the availability of synaptic serotonin (5-HT). However, several findings support the contention that certain SSRIs can block other monoamine transporters, such as those implicated in noradrenergic transmission.

AIM: Build a model to test how SSRIs such as fluoxetine influence the noradrenergic system.

Research Plan/Model (10 points)

- To test how SSRIs such as fluoxetine can influence the noradrenergic system, I need to build a model that can mimic the cytoarchitecture of the noradrenergic circuits. Acute brain stem slices obtained from wild-type mice are a model rich in noradrenergic terminals since the locus coeruleus, which is in the pons area of the brain stem, is one of the main sites of the noradrenaline innervation.
- Fresh brain stem slices of about 250 μm of thickness maintained in complete cell media, will be treated with increasing concentration of fluoxetine and spontaneous firing rate of noradrenergic neurons will be measured through electric recordings. If SSRIs influence noradrenergic transmission, a decrease activation of noradrenergic neurons should be recorded.

- I chose this model because brain slices provide the same cyto-organization of the tissue/organ from which they are derived, compared to dissociated cells in cultures and it is easier to selectively record firing potentials by noradrenergic neurons.
- Brain Stem dissociated cultured neurons do not retain tissue cytoarchitecture and present a larger variability in terms of type of neurons.

[1]Celada P, Puig M, Amargós-Bosch M, Adell A, Artigas F. The therapeutic role of 5-HT1A and 5-HT2A receptors in depression. *J Psychiatry Neurosci*. 2004;29(4):252-265.

Part 2

- In this project, Mr. B investigates the role of fluoxetine on the noradrenaline system using brain slices. Brain slices will be obtained cutting the mouse brain at the level of the locus coeruleus, an area specific for the noradrenergic pathway.
- The choice of brain stem slices to study the effect of fluoxetine on the noradrenergic pathway appears to be the most appropriate method since it allows to investigate the direct effect of the administration of this compound on the target. Other models cannot be considered since some do not preserve the brain's 3D organization (such as cell cultures) while others are not at an advanced stage of development enough to mimic brain's cytoarchitecture (such as brain organoids).
- Unfortunately, with brain slices drug treatment can only be done in acute, since slices are only viable for a short amount of time.
- Thus, to study long term effect on fluoxetine on the noradrenergic system, the use of animal models such as wild-type mice are recommended. Mice can be treated on a sustained regiment of this drug for a longer period and then noradrenergic signaling can be investigated by specific pharmacological inhibition and behavioral tests in live animals.