**Specific Aims**

Major depression is a severe mental illness that has debilitating effects on an individual’s daily life and is a major contributor to early mortality due to suicide [1]. One of the many factors that increase susceptibility to depression is genetics. Recent work has identified two single nucleotide polymorphism (SNPs) near the Myocyte Enhancer Factor 2C (MEF2C) gene locus in individuals of European and Australian descent with depression [2,3]. MEF2C is a transcription factor that regulates the density of excitatory and inhibitory synaptic neurons to refine synaptic connections during the development to the brain [4]. Transcriptional activation of MEF2C is thought to be dependent on phosphorylation by Extracellular-signal-regulated kinase 5 (ERK5) and p38 mitogen-activated protein kinase (p38 MAPK) and this pathway is important in neurotrophin-promoted survival of developing neurons [5]. *Yet,* *little is known about how phosphorylation of MEF2C affects its role in regulating synaptic density in depression*. Understanding how phosphorylation mediates MEF2C in regulation of synaptic density may contribute to the development of therapeutics for depression and associated diseases that affect many people worldwide.

My **primary goal** is to understand the role of phosphorylation on MEF2C in the regulation of synapse elimination during brain development. I will use zebrafish because depressive behaviour and brain function is easily observed [6]. My **hypothesis** is that mutation of conserved phosphorylation sites in MEF2C will result in improper elimination of excitatory neurons, leading to an imbalance in the number of excitatory and inhibitory neurons. My **long term goal** is to gain a better understanding of how defects in MEF2C regulation affects its downstream targets in organizing synaptic density, leading to depression.

**Aim 1: Identifying conserved phosphorylation sites across MEF2C homologs involved in neuronal development**

**Approach:** I will use MEGA and CLUSTAL OMEGA to do sequence alignment of the MEF2C protein in homologs obtained using ENSEMBL to identify evolutionarily conserved phosphorylation sites. I will then do a screen using zebrafish by mutating these phosphorylation sites using CRISPR/Cas9 and identify mutants with behavioral phenotypes indicative of depression. The brains of individuals with depressive phenotypes will be examined with immunohistochemical markers for excitatory and inhibitory neurons to measure synaptic density.

**Rationale:** Mutations in conserved phosphorylation sites in species with complex nervous systems will affect transcriptional activation of MEF2C, resulting in abnormal elimination of excitatory synaptic neurons.

**Hypothesis:** Zebrafish with mutations in conserved phosphorylation sites will have phenotypes associated with depression and abnormal synaptic density since MEF2C regulates neuronal development.

**Aim 2: Identifying differently expressed genes important in maintaining synaptic density in development**

**Approach:** I will isolate mRNA from brain tissue of phosphorylation mutant MEFC2 zebrafish (from Aim1) and wildtype (WT) individuals and conduct RNA-seq. I will then identify differentially expressed genes (DEGs) between mutants with and without depressive phenotypes and classify the functions of these genes using Gene Ontology. DEGs involved in neuron development will be used in a gene set enrichment analysis to identify statistically significant correlations between these differences and depressive phenotypes. I will conduct a loss of function screen with CRISPR/Cas9 using these statistically significant DEGs and examine synaptic density in WT and mutant brains using immunohistochemistry.

**Rationale:** Genes involved in neuron elimination and development will be differentially expressed as they might not be transcriptionally activated or repressed by mutated MEF2C. Understanding the expression profiles and functions of these genes allows us to recognize the pathways linked to neuron development and how they are regulated by MEF2C.

**Hypothesis:** Zebrafish mutants that display depressive phenotypes have different gene expression profiles for genes involved in neuron development and migration compared to mutants without depressive phenotypes and WT individuals.

**Aim 3: Identifying kinases responsible for MEF2C phosphorylation important in synaptic density**

**Approach:** I will conduct pull-down/mass spectrometry using tandem affinity purification (TAP)-tagged baits made from regions of MEF2C containing conserved phosphorylation sites. Bait-prey complexes are allowed to form in protein lysates prepared from brains of WT and mutant zebrafish. Mass spectrometry is then used to identify these interacting proteins and Gene Ontology is used to assess the functions and identity of these proteins, specifically looking for kinases that bind to WT but not mutant MEF2C. I will use CRISPR/Cas9 to conduct a loss of function screen for these kinases and measure synaptic density in WT and mutant brains using immunohistochemistry to validate the role of these kinases in synaptic density regulation.

**Rationale:** Identifying proteins that interact with the regions of MEF2C containing phosphorylation sites will allow us to understand which specific kinases interact with MEF2C to regulate synaptic density. A comparison of kinases found in WT and mutant zebrafish will reveal kinases that are important for synaptic density regulation.

**Hypothesis:** Kinases that bind to WT MEF2C but are unable to bind to mutant MEF2C are important in the phosphorylation of MEF2C in regulating synaptic density.

Identifying how phosphorylation affects the role of MEF2C in regulating synaptic density balance is important in establishing a biological pathway for depression. This will allow the development of therapeutics aimed at rebalancing synaptic density in patients with depression due to defects in MEF2C phosphorylation.

**References:**

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