Biomedical Application #1

Research Description:
Impact of radical prostatectomy and androgen deprivation on penile fibrosis, smooth muscle and erectile function

Significance: Annually, one in seven men are diagnosed with prostate cancer and are often treated pharmacologically with androgen deprivation therapy (ADT) to shrink the prostate followed by a radical prostatectomy (RP). A common side effect of this cancer treatment is erectile dysfunction (ED) which severely impacts the quality of life of these cancer survivors. Testosterone is known to prevent fibrosis which can impact the ability of the penis to relax. Fibrosis is caused when the end organ becomes hypoxic due to nerve injury as a result of RP. Restoring blood flow and promoting neuronal survival or regeneration can prevent penile fibrosis which is a leading cause of ED. The impact of both ADT and RP combined on erectile function and fibrosis has not been examined.

Purpose: To assess impact of neoadjuvant ADT and pelvic nerve injury on erections, penile smooth muscle function, and penile fibrosis.

Hypothesis: We hypothesize that there will be an additive effect among ADT and RP leading to an increase in fibrosis, and a decrease in both smooth muscle and erectile function.

Methodology: The four groups will be 1) sham, 2) castration, 3) bilateral cavernous nerve injury (BCNI), and 4) castration and BCNI (n = 8/group). In order to mimic ADT, animals will be surgically castrated. Four weeks post castration, to mimic a radical prostatectomy, animals will undergo BCNI. Apomorphine induced erectile responses and yawns will be measured weekly at baseline, after castration, and following BCNI surgery. After euthanasia, penile tissue strips will be mounted in a muscle strip myograph. Contractile and relaxation responses to phenylephrine, endothelin-1, thromboxane, and acetylcholine will be measured. Histological staining will be performed using trichrome and picrosirius red to assess smooth muscle and collagen composition.

Preliminary Results: A pilot study in our lab, tested two animals from each group. My role was to record and analyze the apomorphine test responses. Additionally, the contractility of penile strips was also measured. We saw that erectile function was the most decreased in that rats who had undergone castration and BCNI. This preliminary data supports our hypothesis.

Researcher’s Role: My role will be to perform apomorphine testing, assist in analyzing smooth muscle contractility using the myograph, and perform histological analysis. I have been working in Dr. Hannan’s lab for just over a year and have become proficient at performing apomorphine tests, analyzing neuronal survival, and more. My neuronal culture work has already resulted in a publication where I am a co-author. I am currently learning how to analyze smooth muscle physiological testing and to perform and analyze histological staining.

Budget Justification:
Dr. X has start up money which covers the animals and basic supplies. The lab has an approved AUP (Q343) for rodent subjects. I have completed ECU’s Animal Care and Use Committee rodent handling certification. We are requesting funds specific to the smooth muscle and erectile function analysis.
Histology
- Trichrome Staining Kit $309.00
- Picrosirius Red Kit $346.67
- Xylene histology $127.00
- Alcohol $20
- Hematoxylin $130.00
- Eosin $250.43
- Alpha Smooth Muscle Antibody $405.00
- Tissue Processing Cost (ECU Histology Core) $320.00

Myograph
- Endothelin 1 $247.50
- Thromboxane $139.50

Total: $2,295.10

Biomedical Application #2

Research Description:

Hypothesis: Autism brain organoids will form fewer inhibitory synapses, resulting in increased formation of excitatory synapses.

Purpose/Significance: Autism is one of the fastest growing developmental disabilities, currently affecting 1 in 58 children in North Carolina (Christensen et al., 2016). Thus, there is a pressing need to understand the molecular mechanisms leading to the development of Autism. Autism is a cognitive disorder characterized by social deficits and the presence of restricted and repetitive patterns of behaviors or interests. At a cellular level, post-mortem patient brains exhibit increased excitatory synapses (Tang et al., 2014; Yi et al., 2015; Hutsler and Zhang, 2010). The underlying mechanisms for this increase are still unclear. However, decreased inhibitory signaling may affect synaptic refinement. In order to capture synapse formation during early brain development, we culture cortical brain organoids from Autism patients. Importantly, these organoids resemble mid-fetal brain development, approximating in utero human brain development at 19-24 weeks of gestation (Paca et al., 2015). This developmental stage coincides with the predicted emergence of Autism pathology during the second trimester (Stoner et al., 2014). Thus, cortical organoids provide a unique opportunity to observe the development of Autism pathology and to test our hypothesis that decreased inhibitory synapses result in increased excitatory synapse formation.

Preliminary Results: Our Autism-derived cortical organoids exhibit increased excitatory synapse formation. These Autism-derived organoids, together with neurotypic controls, will be used to assess whether Autism brain organoids have fewer inhibitory synapses than neurotypic controls.

My Project Role/Methodology: I will identify synapses by immunofluorescence staining of brain organoids for the inhibitory synaptic marker, gephyrin, and the excitatory synapse marker, vGlut-1. I will
then image these samples on a confocal microscope, followed by analysis of the density of inhibitory to excitatory synapses. I will also perform a timecourse of neuronal differentiation to determine when inhibitory and excitatory synapses form and when alterations between Autism and control-derived neurons occur during neurodevelopment. My future research will determine whether decreased inhibitory signaling promotes increased excitatory synapse formation. I will treat control brain organoids with an inhibitor of the ion channel, KCC2, which mediates inhibitory signaling (Sivakumaran et al., 2015). I will then determine whether this treatment results in increased excitatory synapses similar to Autism by using confocal microscopy and analysis of inhibitory and excitatory synaptic densities. These studies will determine whether increased excitatory synapse formation in Autism is a result of decreased inhibitory signaling or whether it results from mechanisms that specifically alter excitatory synapses.

**Budget Justification:**

I am requesting the funds for primary antibodies, secondary antibodies, and lab equipment so that we will be able to stain and analyze the different neuronal markers in the 3-D cortical models. These will help in studying more about the development of these synapses and help in analyzing the different types of neuronal markers that will be affected by these brains.

**Substance Price**

**Primary Antibody:**

Chicken polyclonal Anti-Double Cortin Antibody (500uL) 389 (Abcam ab153668)

Mouse monoclonal Anti-Gephyrin Antibody (100ug) 415 (Synaptic Systems 147-011)

Guinea Pig polyclonal Anti-VGLUT1 Antibody (100uL) 330 (Synaptic Systems 135-304)

**Secondary Antibody:**

Goat Anti-Guinea Pig IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (Invitrogen A-21450) (500uL) 209

Goat Anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 568 (Invitrogen A-11004) (500uL) 169

Goat Anti-Chicken IgY (H+L) Secondary Antibody, Alexa Fluor 488 (Invitrogen A-11039) (500uL) 169

**Equipment:**

Flouro â€” Gel II Mounting Medium 100mL 270 (Electron Microscopy Sciences 17985-51)
Total: 1,951