**[PROJECT #1] Developmental programming of skeletal muscle: impact of fetal growth retardation on muscle morphology in adult rats**

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The early-life environment, particularly in the fetus, is now recognised as an important determinant of several adult-onset diseases. Thus, increased frequencies of hypertension, obesity, dyslipidemia and type 2 diabetes, collectively referred to as the ‘Metabolic Syndrome’, have been linked to intrauterine growth retardation (IUGR), an assumed marker of a poor fetal environment. These links are thought to reflect ‘developmental programming’, the process by which various organ systems are affected during specific developmental periods of susceptibility such that consequent pathological effects emerge later in life.

In our programming model of fetal glucocorticoid excess in the rat, we see consistent programming of high blood pressure, reduced insulin sensitivity in adult offspring (ref 1) and aberrant skeletal muscle gene expression (ref 2). Specifically, fetal glucocorticoid exposure programmed for elevated glucose transporter 4 (Glut4) and decreased uncoupling protein 3 (UCP3) mRNA in the gastrocnemius muscle of offspring at 6 months of age.

**AIM:** This project aims to identify whether gastrocnemius morphology, in terms of myofibre crosssectional size or proportion of fast to slow twitch fibres, is altered by fetal glucocorticoid excess.

**PROJECT:** The task for this project is to investigate changes in the size and type of muscle fibres in the superficial gastrocnemius muscle from offspring of glucocorticoid-treated mothers. All tissues have already been collected; students will be required to cut, stain and conduct stereological analyses on these muscles. Histochemistry with NADH-TR will be used to differentiate fast and slow type myofibres within the gastrocnemius muscle (ref 3).

Comparisons of fast and slow twitch fibres will be made for control and glucocorticoid-exposed offspring. Comparisons of myofibre size will be made using two different methods:

1. Unbiased stereological techniques to determine the average myofibre crosssectional area
2. Image Pro analysis by outlining individual myofibres to calculate crosssectional areas

**References:**

PROJECT #2  Is there influx of macrophages due to voluntary exercise?

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Background: Macrophages are immune cells which are derived from circulating monocytes and are part of the innate immune defence system. Presence of macrophages is of critical importance not only during disease but also in maintaining tissue homeostasis within muscle. For example lack of macrophage presence has been associated with decreased muscle regeneration (1-4). This project aims to investigate if voluntary (moderate) exercise increases influx of macrophages (i.e. bone-marrow derived monocytes) (4-6).

To study the recruitment/influx of macrophage precursors (i.e. monocytes) into muscle tissue, we have employed transgenic CX3CR1+/−/GFP (aka CX3CR1−/−) mice. The latter mice express CX3CR1, a monocyte-derived cells marker, on one allele and green fluorescent protein (GFP) on the other allele. Thus, all cells of monocytic lineage express GFP. We have used these mice as bone marrow donors to rescue the immune system of ‘whole body’ irradiated wild-type mice. As a result, all donor bone marrow-derived cells of monocytic lineage (i.e. macrophages and dendritic cells) that infiltrate tissues can be tracked and identified by the GFP label. In the muscle, these donor-derived cells can be distinguished from host macrophages by expression of both GFP and IBA1 (macrophage marker) [GFP+/IBA1+] as opposed to resident macrophages that express only IBA1 [GFP−/IBA1−].

To investigate influx of macrophages into muscle tissue due to exercise the chimera mice have been given the opportunity to exercise within three different environments (i.e. control cage, control cage + running wheel, ‘enriched environment’) for a period of 8 weeks.

This study aims to compare the number of infiltrated macrophages (GFP+) in muscles between mice from the different environments (n=4 per group). In addition, total number of macrophages will be determined using IBA1 antibody. Muscle samples have been collected and cut and students will be required to immunostain the sections and perform stereological analysis to determine the proportion of infiltrated macrophages.

References:
PROJECT #3 Astrocyte morphology following partial optic nerve injury in a model of secondary degeneration

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Secondary degeneration is a form of ‘bystander’ damage that can affect neural tissue both nearby and remote from an initial injury. Partial optic nerve transection is an excellent model in which to unequivocally differentiate events occurring during secondary degeneration from those resulting from primary CNS injury 1. Using this model we have recently demonstrated that the CNS-specific L- and T-type calcium channel blocker lomerizine alleviated some of the events implicated in progression of secondary degeneration 1.

Injury to the CNS also results in dramatic changes to astrocytes, including migration towards the injury site and hypertrophy 2,3. As little as five minutes after partial transection of the optic nerve we have observed qualitative changes in astrocytes including cell swelling in areas of the optic nerve vulnerable to secondary degeneration. In this project students will quantify the changes in astrocyte morphology from immunohistochemically stained tissue sections obtained from animals 5 minutes, 3 hours, 24 hours or 3 days after partial transection 4. Sections from animals treated with lomerizine for 24 hours will also be assessed. The following hypotheses will be examined:

1) Astrocyte morphology will alter in areas of the optic nerve vulnerable to secondary degeneration in the 3 days following partial transection

2) 24 hours of lomerizine treatment will modulate the observed changes in astrocyte morphology.

References

PROJECT #4 Histological differences in the spleen of FKN-Rc deficient mice in comparison with wild type mice

*Academic consultant* Luis Filgueira

The chemokine fractalkine and its receptor are expressed on immune cells. However, fractalkine receptor deficient mice seem to have a normal immune system. This project is about comparing the spleens from these mice with wild-type mice using histological methods.

*Question:* Do fractalkine deficient mice have a different histology of spleen, in comparison with wild-type mice.

*Aims of the project:*
Study the histology of the spleen of fractalkine receptor deficient mice and compare it with that of wild-type mice.

*Hypothesis:*
Fractalkine receptor deficient mice have a different histology of the spleen in comparison with wild-type mice, although there seems to be no difference in the immune function.

*Experimental setting:*
The spleens of 5 fractalkine receptor deficient and wild-type mice will be processed for histology and analysed.
The role of CX3CR1 signalling in the differentiation of mast cells in the eye.

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Cx3cr1 is the sole receptor for the chemokine fractalkine (Cx3cl1). Cx3cr1 is expressed by a wide range of immune cells including macrophages, dendritic cells, T cells, NK cells and also mast cells (Amr El-Shazly et al. 2006, Journal of Immunology, 176:1860-1868). In the mouse cornea, DCs are unable to populate the corneal epithelium in the absence of Cx3cr1 signalling (Chinnery et al., 2007, IOVS, 48: p1568). The choroid is found in the uveal tract of the eye. This vascular layer contains dense populations of macrophages, DCs and mast cells. This project would involve comparing the distribution and density of mast cells in the choroid of young mice which either have normal expression of Cx3cr1 or are lacking Cx3cr1.

We have WT (n=6), heterozygous (n=5) and homozygous (n=9) (cx3cr1-deficient) albino tissue (Balb/c CX3CR1-gfp mice) that students could process for paraffin (or resin) sections and wholemounts to compare the numbers of choroidal mast cells. These techniques could illustrate the difficulty in locating widely dispersed cells using conventional sectioning Vs wholemount methods.
**Aim:** To determine when different developmental proteins are switched on/off during skeletal muscle regeneration

**Background:** Mutations in skeletal muscle α-actin cause multiple, mostly severe and currently incurable muscle disorders (Nowak et al., 1999). Cardiac α-actin is the predominant actin isoform in foetal skeletal muscle (Ilkovski et al., 2005), but is down-regulated by birth, and replaced by skeletal muscle α-actin (Vandekerckhove et al., 1986). Furthermore, cardiac α-actin is the principal actin isoform expressed during skeletal muscle regeneration (Moll et al., 2006). A possible treatment for skeletal muscle α-actin diseases is replacement of skeletal muscle α-actin with cardiac α-actin in adult skeletal muscle. If we can determine when and how the switch between these two isoforms occurs, then this information could be used to establish an effective therapy for skeletal muscle α-actin diseases.

For this study, expression of cardiac α-actin and skeletal α-actin (as well as other developmental proteins) will be examined in a muscle regeneration model, the whole muscle graft (White et al., 2000). Transplantation of extensor digitorum longus (EDL) muscles causes necrosis of muscle fibres throughout the length of the graft, and a pattern of regeneration is observed that occurs progressively from the periphery of the muscle towards the necrotic centre.

**Tissue:** EDL muscles collected from mice which have undergone whole muscle graft surgery Day 0, 5 days post surgery, 10 days post surgery

**Techniques and Analysis:**
- Cryostat sectioning
- Fluorescence staining and analysis of sections
- Light microscopy of stained sections

**References:**
**PROJECT #7**  The effect of an orally-bioavailable compound on expression of foetal proteins in mouse skeletal muscle

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**Background:**  
Skeletal muscle is crucial for respiratory function and everyday activities. It comprises 40% of the body and thus diseases affecting the skeletal musculature can be very debilitating and in some cases life-threatening. Our research focus is to investigate potential therapies for genetic muscle disorders including Duchenne muscular dystrophy (DMD) and nemaline myopathy (Nowak et al. 2007). These diseases arise due to mutations in the genes encoding dystrophin and skeletal muscle alpha-actin, respectively. One potential therapeutic approach for these diseases is to up-regulate an alternative form of these proteins in order to compensate for the absence of the particular protein or the presence of a disease-causing mutant protein.

It has been shown that up-regulation of utrophin (the foetal form of dystrophin) is able to ameliorate the disease phenotype of the *mdx* mouse (the mouse model of DMD) and the Golden Retriever Muscular Dystrophy dog (Tinsley et al. 1996), (Cerletti et al. 2003). Similarly we have shown that up-regulation of cardiac alpha-actin (the foetal form of skeletal muscle alpha-actin (Ilkovski et al. 2005)) can rescue the early lethality of skeletal muscle alpha-actin knock-out mice (Nowak et al, unpublished data). Additionally, we hypothesise that the foetal form of the muscle glycogen phosphorylase enzyme, so-called “brain” glycogen phosphorylase might be able to alleviate the symptoms of McArdles disease (caused by defects in the muscle glycogen phosphorylase gene). Given that up-regulation of these foetal proteins is effective in alleviating the disease severity in these animal models, our aim is to identify a compound that is capable of inducing expression of these foetal proteins (cardiac alpha-actin, utrophin and brain glycogen phosphorylase) in adult, mature skeletal muscle.

A limited preliminary study has identified a compound (compound A) that is capable of inducing expression of cardiac alpha-actin and brain glycogen phosphorylase in adult skeletal muscle. In order to further investigate the effect of this compound on protein expression, we have been administering compound A in the drinking water to a group of six 10-week old mice. We also have a control group of mice that are receiving the same drinking water but without Compound A as the untreated group.

**Aim:**  
The aim of this study is to examine the expression of foetal proteins (cardiac alpha-actin, utrophin and brain glycogen phosphorylase), using immunohistochemistry, in skeletal muscle from treated and untreated mice.

**References:**  
Characterisation of hepatospheres comprising mesenchymal and liver progenitor epithelial cells

Brief outline:

Liver progenitor cells are characterised by their ability to differentiate into hepatocytes and cholangiocytes. They are derived from mixed cultures of mesenchymal cells and liver epithelial cells from fetal liver. In the long term, it is possible to derive pure liver progenitor cells (LPCs) from such cultures. The mixed culture will generate hepatospheres but not the pure culture. We have preliminary evidence which suggests that the hepatospheres enhance differentiation of LPCs into hepatocytes. This project will test this hypothesis by determining the level and pattern of gene expression of LPCs in the two models by histology and immunohistochemistry.

Contact Prof George Yeoh & Dr Roslyn London
Biochemistry and Molecular Biology  Phone 6488 2986 yeoh@cyllene.uwa.edu.au
Spinal cord injury (SCI) results in disconnection of descending motor systems and ascending sensory pathways, leading to motor dysfunction and paralysis below the lesion – with very little regenerative response occurring in the adult mammalian CNS. Important factors crucial for the effective repair of the injured spinal cord (SC) include a reduction of cell death and secondary injury cascades in the SC after the initial impact as well as the promotion of tissue sparing, axon regeneration and possibly cell replacement at the injury site. To date, a number of cellular transplantation approaches have been used to address these issues (reviewed in 1). Bone marrow stromal (stem) cells (BMSCs) are defined by their capacity for multilineage differentiation into a range of tissues of mesodermal origin, and there is great interest in potential therapeutic application of BMSC 2 to spinal cord injury (SCI).

Studies have reported the potential of BMSCs to differentiate into cells with many of the phenotypic characteristics of neural tissue 3,4 and the capacity to migrate 3 and integrate into CNS tissues and express markers typical of mature neurons and astrocytes 4. Significantly, BMSCs have also been successfully transplanted into the mammalian spinal cord and reported to: (a) promote regeneration of lesioned axons into the graft 5, (b) differentiate into neurons 5, (c) remyelinate damaged myelin sheaths around CNS axons 6, and (d) improve the functional outcome after SCI 5,7. The use of autologous donor cells in any clinical setting has advantages over allogeneic populations in addressing immune rejection. Importantly, this study uses donor hBMSCs from SCI patients, since the use of autologous hBMSCs from injured patients 8 will be preferred in any transplantation therapy. To date, isolated and highly purified hBMSCs from SCI patients have not been directly applied to SCI as a therapy. A few studies have used partially purified donor hBMSCs 9, 7 in rats and also in humans 8, 10, but none of these observations have so far been attributed to the cell therapy itself.

Our novel repair strategy for SCI uses such highly purified (Stro-1+) multipotent hBMSCs 11 isolated from SCI patients engineered to express enhanced green fluorescent protein, GFP (hBMSCGFP) which and are transplanted into immunologically deficient (Nude) rat hosts (either with or without further immunosuppression) that have been subjected to either an acute (1 wk) or chronic (1mo) a moderate contusion injury, resulting in a marked improvement in functional (behavioural) recovery. Most new SCI patients (acute) will eventually become chronic, and so clinical applications must target chronic injuries. This project will assess whether hBMSC treatment promotes tissue sparing (ie a reduction in the loss of tissue at the injury site) after acute/chronic injury in order to further support the potential for clinical application of hBMSC therapy to SCI.

This project will involve morphological assessment of tissue damage and cyst size in spinal cord sections already taken from acute and chronic injured rats subjected to hBMSC treatment. Spinal cord sections will be histologically stained and images subjected to quantitative measurement using software that will allow us to compare hBMSC treatment between acute vs chronic injury.

References:
7 Neuhuber, B. et al., Brain Research 1035 (1), 73 (2005).
Does the tasmanian devil eye display any curious adaptations to its lifestyle and evolutionary history

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We have wet fixed tissue available and embedded blocks in both parafin and resin. Students could look into two main issues. A. Do devils have the double retinal vessels seen in all other marsupials with retinal blood supply? B. Does the cornea display any obvious adaptations that reflect the trauma that devils appear to suffer from their aggressive behaviour.
Lead exposure has increased in the Australian population, especially in lead mining and processing areas. Bone is the major tissue of lead accumulation in the human body. Osteoclasts, the only bone resorbing cells must be exposed to higher concentrations of lead. However, it is not known whether lead has any effects on osteoclast differentiation and functions.

*Question:* Does lead influence osteoclast differentiation and function.

**Aims of the project:**
The aim of this study is to investigate the effects of lead on human osteoclasts *in vitro.*

**Hypothesis:**
Increasing lead concentrations to toxic amounts found in lead poisoned individuals, changes osteoclast differentiation and function.

**Experimental setting:**
Human osteoclasts will be cultured from monocytic blood cells and incubated with different concentrations of lead. Fluorescence microscopy methods will be used for the detection of lead-related changes in osteoclasts.
PROJECT #12 Changes in ageing muscle

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Background: A very large proportion of the elderly (>65 years) suffers from muscle loss (i.e. sarcopenia). As a result the elderly person loses muscle strength to control movement causing imbalance and susceptibility to fall and fracture which can have devastating effects. To understand the underlying mechanisms causing sarcopenia and to be able to design appropriate interventions, we need to understand the changes occurring in muscle over time (1-4). Using aged mice up to 30 months old (see table below; mouse life-span 2-3 years) we aim to characterise changes between young and old mouse muscles in terms of:

- Total size of the muscle in cross-section
- Total number of myofibers
- Proportion of fast and slow myofibres
- Size of fast and slow myofibres

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A portion of the material has been sectioned while of other will need to be done. In addition all sections are required to be stained with H&E to visualise general morphology and NADH to identify the myofibre type. Next selection of sections will be scanned and stereological analysis conducted.