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THE WINSTON CHURCHILL MEMORIAL TRUST OF AUSTRALIA

Report by - BRYAN LEAW - 2016 Churchill Fellow

THE DOROTHEA SANDARS CHURCHILL FELLOWSHIP
to study amnion epithelial cells as a therapy for
cerebral palsy

Signed Dated October 2017

Key words: cerebral palsy, neuroscience, stem cells, cell therapy, translational research,

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INTRODUCTION

Cerebral palsy describes a spectrum of motor impairments that may include associated intellectual or speech impediment. Strikingly 17 million children worldwide have cerebral palsy, and in Australia, every 15 hours a child is born with cerebral palsy. Despite improvements in clinical management, there is no cure for cerebral palsy. Stem cell therapy has shown promise in experimental models of disease, and we believe a particular type of stem cell, the human amnion epithelial cell, may herald a new generation of therapies for CP. However, we need validation from experimental animal models of disease before progressing our research into first-in-human clinical trials.

The Winston Churchill Foundation supported several aims for my Fellowship:

1) To develop collaborations in both Canada and the United States for my growth as a future leader in my field;
2) To be trained in a world-leading laboratory for cerebral palsy research in the development of a rabbit model of hypoxic-ischemic encephalopathy – one of the major events thought to underlie the development of cerebral palsy.

Professor Sidhartha Tan and his group at the Wayne State University / Children's Hospital of Michigan lead ground-breaking research into the pathways leading to the motor symptoms seen in cerebral palsy, as well as translating these findings into human clinical trials. The skills and expertise in his lab are unique worldwide, and this fellowship enables me to bring these back to the Ritchie Centre at the Hudson Institute of Medical Research – a leading Australian maternal and perinatal research group. Crucially, this model of cerebral palsy mirrors many of the motor deficits seen in human cerebral palsy, suggesting that it shares many of the disease mechanisms seen in humans. This presents us a powerful technique to test new and novel therapies. This model will be one of many maternal and perinatal disorders we wish to set up in partnership with Monash Health – home to one of the busiest obstetric wards in Victoria. We intend for this model to be one of many for a platform of pregnancy and infancy disorders and to lead the country nationally in translational research.

ACKNOWLEDGEMENTS

First and most importantly, I would like to offer my heartfelt gratitude to both the Winston Churchill Memorial Trust, and to Dr Dorothea Sandars, this work would not be possible without their support. This unique opportunity enabled me to work with leading international laboratories, and establishes invaluable collaborative partnerships. Without a doubt this has strengthened my position as a future leader in the field.

I would also like to thank Professor Euan Wallace and Dr Rebecca Lim who are my supervisors back in the Ritchie Centre, based at the Hudson Institute of Medical Research. Their continuous support and guidance meant this fellowship was not possible without them.

I would like to thank Professor Sidhartha Tan for sharing his knowledge and immense experience in the field of cerebral palsy research. Despite only recently moving to Wayne State University, the magnitude of translational research and cutting edge equipment available at his disposal meant I learnt and grew a great deal since the beginning of my Fellowship. Of particular note, while sounding benign, was the filing system used to track data files of the numerous projects and collaborators occurring simultaneously in the laboratory – I will certainly look to implement this back in Melbourne.

Dr Zhongjie Shi and Dr Kehuan Luo were Professor Tan's postdoctoral officers who mentored me on a day-to-day basis, ensuring I could find my way around the lab and have a fruitful few weeks in the surgery suites. Without a doubt my time in Detroit would not be nearly as fun and productive without them around.

I am also grateful for the support and assistance of the other laboratories that were generous enough to meet with me and show me around their facilities. These include, in no particular order: Caroline Dunk (Luenenfeld Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada), members of Dr John Kingdom's Laboratory (Luenenfeld Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada),

EXECUTIVE SUMMARY

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PROJECT DESCRIPTION

The purpose of my Dr Dorothea Sanders Churchill Fellowship was to ascertain whether human amnion epithelial cells could be used as a novel therapy for cerebral palsy. To do so, I visited Professor Sidhartha Tan's laboratory at the Children's Hospital of Michigan, Wayne State University, Detroit, USA to learn a world-leading animal model of cerebral palsy. The intentions of this visit were to be trained on developing this model back in Australia, as well as establishing new collaborations between two leading research groups.

HIGHLIGHTS

- ☐ Being trained on and exposed to the intricacies of a world-renowned disease model
- ☐ Meeting world leaders in the cerebral palsy and pre-eclampsia research fields.
- ☐ Exposure to world-class clinical and laboratory facilities, being trained on cutting-edge equipment not available back in Melbourne.
- ☐ Developing collaborative networks to submit international grants.

LESSONS LEARNED

- ☐ Development of the rabbit model of cerebral palsy will require significant upgrades to the facilities available back in Melbourne. This trip gave me a vital in-depth understanding of how to perform the surgery and what changes need to be made.
- ☐ Unexpectedly, whilst on a meeting at Mount Sinai, Toronto, I met a PhD student from a maternal research group who was developing a model of pre-eclampsia that our laboratory has been interested in. I received vital advice on developing the model and received the contact details of the trainer who will train us.
- ☐ Optimisation of common molecular lab assays as well as better ways of organising data storage. The advances in assay technology available here certainly merit introduction back in Melbourne. Developed strategies for equipment grant EOIs.

OFFICIAL PROGRAMME

START DATE	END DATE	CITY, COUNTRY	INSTITUTION VISITED ADDRESS
11 August	15 August	Edmonton, Alberta Canada	University of Alberta Edmonton, AB T6G 2E1
15 August	20 August	Toronto, Ontario Canada	Lunenfeld-Tanenbaum Research Institute Mount Sinai Health System 600 University Avenue Toronto, ON M5G 1X5
20 August	15 Sept	Detroit, Michigan USA	Children's Hospital of Michigan – DMC 4201 St Antoine St Detroit, MI 48201
15 Sept	22 Sept	New York, NY USA	Weill Cornell Medical College 1300 York Ave New York, NY 10065

CEREBRAL PALSY

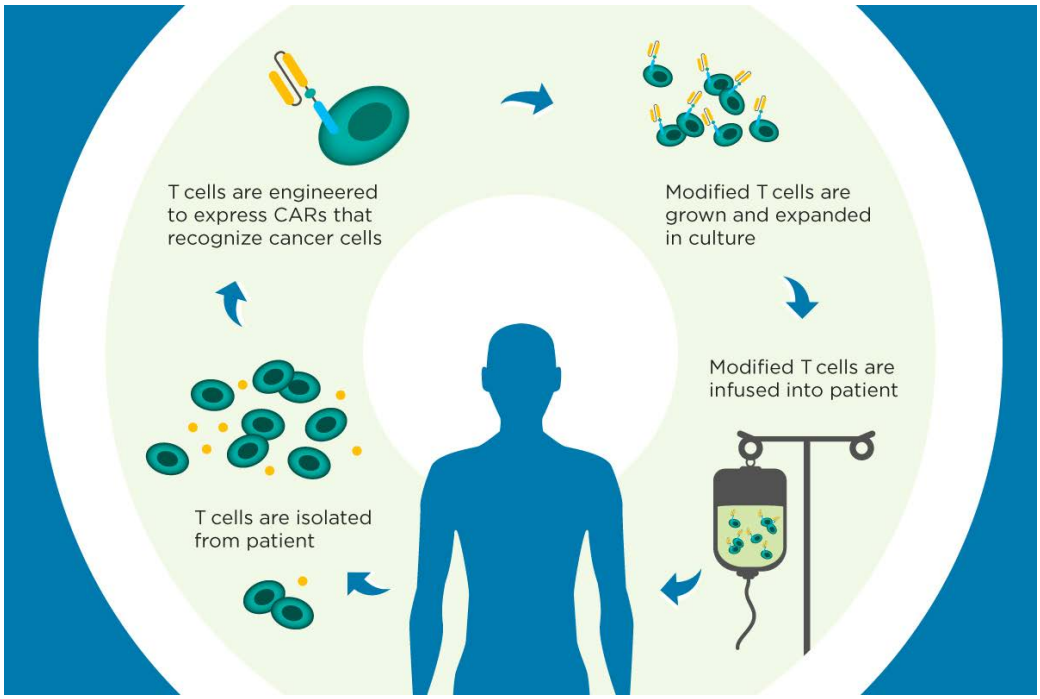
Cerebral palsy (CP) is a lifelong condition characterized by motor deficits, including lack of posture, hypertonia, difficulty walking and overall impaired motor development. Nearly half of all individuals with CP also have an associated intellectual impairment. These have serious social and economical consequences in Australia, as it is a disease that has a lifelong burden. While advances in obstetric and neonatal care have improved the rate of intact survival and, for the first time, resulted in an apparent fall in the prevalence of CP, the majority of children with CP sustain their injury during pregnancy and not at the time of birth or postnatally [1]. In fact, recent evidence suggests that the early stages of injury manifestation happen before delivery, during delivery, or shortly after delivery. Targeted interventions such as magnesium sulphate or head cooling are not relevant to these children. Instead, current interventions are essentially limited to early detection and interventional therapy [2, 3]. What is really required is a reparative therapy. This is where we, and others, believe cell therapy offers much promise.

Source: Cerebral Palsy Alliance, Australia

CELL THERAPIES

The term 'cell therapies' refers to the use of cellular material, be it whole cells such as immune cells and stem cells, or cellular contents, including proteins, growth factors and genetic material such as RNA and DNA. These cells are usually still living, for example the

injection of immune cells into patients to stave off tumorigenicity (the growth and spreading of tumors) termed **immunotherapy**. Perhaps the most well known form of immunotherapy is Car T Cell therapy. In this form of therapy, the patients own T cells, an immune cell type responsible for identifying and removing invading cells, are isolated, re-engineered to specifically recognize, target, and kill cancer cells, allowed to multiply in culture and re-infused into the patient. These cells then go on to deplete the dysfunctional cancerous cells and improve patient outcomes.



Source: Memorial Sloan Kettering Cancer Centre

The other main principle thought to be behind how cell therapy works is via the introduction of stem cells. These cells have infinite capacity to divide and to grow into various forms of cells that replenish dead or unhealthy cells, or alternatively release proteins and soluble factors that encourage the body to perform this process. Thus this form of cell therapy is referred to as **regenerative medicine**. For example in Parkinson's disease, the clinical symptoms are a result of mass dopaminergic neuron death in a region of the brain called the substantia nigra. The lack of the neurotransmitter dopamine then results in motor impairment, and as a result cell therapies for Parkinson's disease aim to replenish these dopamine neurons to reduce or eliminate altogether the physical disability.

In recent years, both forms of these cell therapies have come to the forefront of experimental treatments due in large part to allogeneic cell therapy, or cell therapy that does not require donor matching in between patients. This is a major advantage as a reparative therapy as it allows for large-scale production of “off-the-shelf” products that could one day replace pharmaceutical alternatives. Our laboratory group, based at the Ritchie Centre, Hudson Institute of Medical Research, specializes in research on a particular cell that can be isolated from the placenta. Termed human amnion epithelial cells, these cells are located on the surface layer of the amnion layer, and can be isolated within a few hours following birth in vast amounts (ranging from 100-300 million cells per placenta). Importantly, following delivery, placenta is usually regarded as waste tissue, and unless requested for by the mother, is discarded. The ability to use tissue that typically is disposed and to turn it into a therapeutic agent thus holds great merit.

HUMAN AMNION EPITHELIAL CELLS

In 2007, the idea that the placenta contained cells that might have regenerative properties was not a new one. Our group experimented with different isolation techniques, eventually optimizing a series of digestion steps that resulted in a clinically usable yield of human amnion epithelial cells. Strikingly, our next experiments paved the way for the promise we now unanimously hold in our amnion cells. Firstly, these cells had anti-inflammatory properties – not a particularly novel finding, as amnion cell patches have been used to improve scar healing in both combat and civilian settings. However, we found that these cells also had therapeutic potential in diseases of chronic inflammation, including bronchopulmonary dysplasia and adult liver fibrosis. Next, us and others in the field identified that these cells had stem cell-like properties, being able to grow and differentiate into numerous cell types and thereby could have a potential in regenerative medicine. Importantly for the translatability of these cells and their progression to clinical trials, we found that these cells also do not display human leukocyte antigen (HLA) class II proteins on their surface. The HLA class II proteins are responsible for presenting antigens on the outside of the surface of the cell, and are a major barrier for the usage of human cells in a cell therapy setting – as they activate the immune response in the patient. Thus the lack of HLA class II expression make amnion epithelial cells a compelling candidate for therapy. To

progress this work however, the appropriate model has to be applied. This is what brought me to Prof Tan's laboratory at Wayne State University.

WHAT CAUSES CEREBRAL PALSY?

Cerebral palsy is widely studied, as we still do not quite understand the intricacies leading to the manifestation of motor and potential intellectual impairment later in life. As such there have been numerous models attempting to replicate the series of steps leading to these impairments, although each model can vary in the eventual behavioural or molecular outcomes. Perhaps the most common antecedent for cerebral palsy is hypoxic-ischemic encephalopathy, which is a form of injury to the brain. During delivery, a complication could occur whereby the oxygen saturation levels in the brain drop below ideal levels, thereby causing metabolic stress. The brain uses a large amount of energy and decreases in oxygen level below optimal result in local inflammation and consequently, attraction of activated immune cells to the area, which perpetuate the injury. However, the body is able to begin adapting to the lowered oxygen condition and begins reducing metabolism. The complication is usually transient however, and when oxygen levels then begin returning to normal, the system is unable to cope with the sudden rise in oxygen and creates toxic reactive oxygen species, and downstream oxidative stress. This series of events, results in what is called a hypoxia (low oxygen) ischemia (as a result of limited blood flow) reperfusion (oxygen returning to normal levels) injury. Prof Tan's laboratory has pioneered and perfected a rabbit model that utilizes this form of injury, and re-creates the motor impairments commonly seen in human cerebral palsy.

When choosing the appropriate animal model, thought has to be given towards their development, growth, and finally similarity in symptoms to the human form of the condition. The right animal model should be able to consistently re-create the injury and activate similar disease pathways, with sufficient precision to be able to be utilized to test new and novel therapies. Rabbits have the advantages of being docile, short gestation and puberty cycles, and have in the past few decades become more and more widely used in the field – hence increased availability of surgical and experimental reagents. Even more relevant for human CP studies, rabbits are perinatal motor developers, similar to humans.

This means that the white matter tract development in rabbits, crucial for movement, occurs prior to delivery, much as humans do. This is in contrast to rodent white matter development, which occurs primarily post-delivery, in particular in the first 14 days of birth. As such, the movement disorders in rabbits exposed to hypoxia-ischemia are remarkably similar to humans, in that their motor symptoms progress from hypotonia (lack of muscle tone) to hypertonia (excess muscle tone). Secondly, this model of CP applies global hypoxia-ischemia, which is more clinically relevant, compared to rodent models that typically apply a unilateral hypoxia-ischemia (only to one side of the brain). Thirdly, their greater size means they are more amenable to imaging techniques such as MRI, which allow identification of white matter deficits with greater fidelity and accuracy, particularly important for correlation to improvements in motor outcomes after treatment.

The use of rabbits however does come with some caveats, including and not limited to: the lack of experienced handlers, the relatively small number of breeding facilities from where these animals can obtained from (compared to rodents), and the lack of experience of most animal research facilities in colony maintenance. Given these disadvantages it was vital for me to be able to visit a facility whereby all these points had been addressed, which would allow me to have a good sense of what was required back at the Hudson Institute of Medical Research.

The research associates and fellows here are very experienced with rabbit work and gave me eye-opening and profound perspectives that I had not seen before. Firstly, the need to work extremely close with the governing bodies on animal surgery and care. In Australia, we have a similar process whereby Animal Ethics Committees assess and approve any animal experimentation before work can begin. However, Prof Tan's laboratory takes it a step further and invites veterinarians to regularly assess their protocol, to come and observe their surgeries (time permitting) and to have open discussions with their committees regularly throughout the year. While all these processes happen back in Australia, it is not done quite to the extent at which it is done here, and in fact fosters an open relationship between both the animal ethics committees and the research groups. Animal care is extremely important in any scientific research, and my time here has certainly opened my eyes to ways we can improve this and how we can streamline our ethics application

processes whilst maintaining the highest standard of care.

The research fellows here were also very helpful in detailing how to properly write up a rigorous and careful methodology of the surgery and planning for the ethics application process. The surgery is a difficult and complicated procedure, usually utilizing three people: the surgeon, the surgeon's assistant, and an anesthetist. Their roles are fixed, and expertise in each of their responsibilities is vital to the success and proper recovery of the animal. The surgeon performs all surgical procedures, practice of proper aseptic technique and instructing the assistant. The assistant ensures the surgeon maintains the sterile field by handing equipment, assisting with gowning up, preparation of reagents and equipment when needed, and alerting the surgeon to any situations which may compromise the sterility or health of the animal. The anesthetist is responsible for calculating the correct dosage of anesthesia for a given animal's weight and condition, monitoring depth of anesthesia and adjusting the perfusion of anesthetic as required, monitoring the vital signs of the animal including heart rate, blood pressure, oxygen saturation and breathing, and monitoring all equipment vital to the success of the surgery. The key to a successful surgery, and smooth recovery of the animal post-operation, is communication and quick observations by each member of the team. When the anesthetist observes that the breathing of the animal is quickening slightly, this suggests that the depth of anesthesia needs to be adjusted, and quick communication to the surgeon to slow on any surgical procedures while the adjustments are made. The total time under anesthesia is also crucial for proper recovery. Balancing surgical precision and speed is tricky, and only comes with years of experience. Working with trained surgeons who have done these techniques thousands of times, I learnt many tips and tricks that will greatly expedite my learning process. Having had some animal surgery experience myself, I found it easy to pick up these tips and tricks and feel like I have grown substantially in this sector of my scientific skills.

I also learnt the nuances of identifying behavioral and anatomical changes that to an untrained observer appear normal. Following distal aortic vessel occlusion, this results in short term uterine hypoxia, which is sufficient to cause brain injury and cerebral palsy-like symptoms in these rabbits later in life.

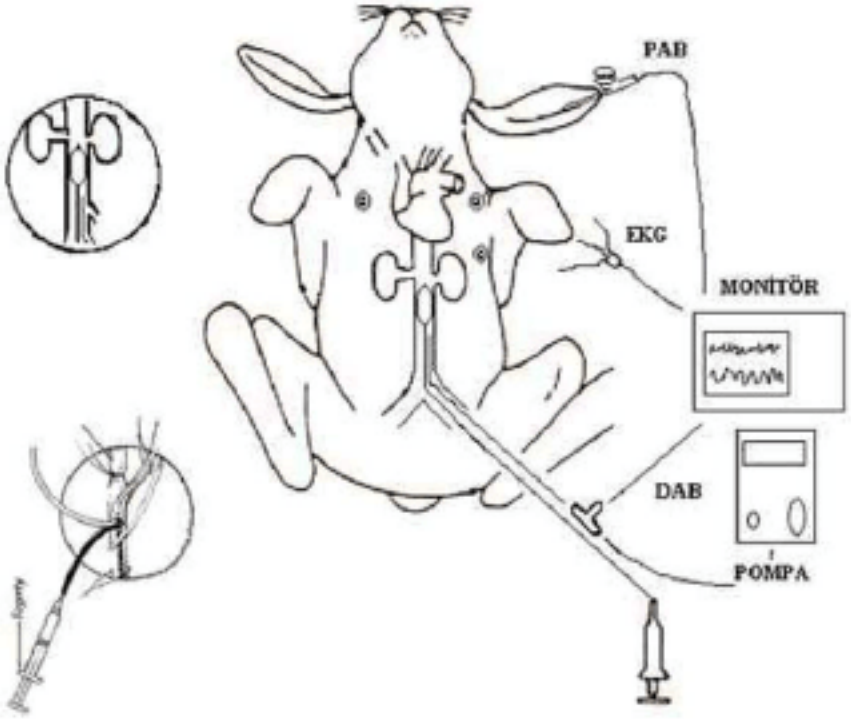


Fig. 1 - The experimental model for aortic occlusion

Illustration of the experimental model, similar to the one used at Wayne State University by Prof Tan's laboratory. The illustration here was taken from Saba et al. [4]. The balloon catheter is inserted via the femoral artery, and pushed through into the distal aorta where the balloon is then inflated. This results in a blockade of the descending aorta, reducing bloodflow and therefore oxygen to the fetuses, reproducing motor symptoms seen in human CP. Animal models can be a powerful tool for medical research, in particular with identifying new novel therapies for human conditions. As can be seen in published work by Prof Tan's laboratory [5], human umbilical cord blood cells significantly reduced the injury seen in these rabbits. This work has now progressed towards late stage clinical trials and represents one of the forerunners for potential cell therapy to treat cerebral palsy. Besides learning the surgery, I also learnt how to perform the behavioural assessments to first distinguish a motor impairment, but also how to identify improvements in motor ability. Using engineering and programming software, we can differences in joint angles during locomotion, and use this data to perform correlations to motor performance. Other assays include the righting reflex, and changes in posture and muscle tone.

During my time here, I also had the opportunity to take on two other side-projects. These

involved the isolation of two different types of placental stem cells from the rabbits, which could potentially lead to the identification and characterization of new therapies. Combining both our molecular and surgical experience, we successfully isolated and cultured these cells and cryopreserved them in liquid nitrogen. This involved isolating the primary tissue from the rabbits, digesting the tissue to pull out single cells, and finally applying a density gradient to isolate these cells. A density gradient is applied by using a series of liquid layers at different densities, from which the cells would get lodged in between following a centrifugation step (separation by centrifugal force in a spinning rotor). We spent substantial time optimizing the right density gradients to be able to elute a pure population of the cells we were interested in, and optimized the digestion steps for the tissue. These could lead to projects in the future where these cells are assessed for their stem cell properties or any immune cell modulating properties. While we ran out of time to do so, we have laid out plans to characterize these cells by using flow cytometry, which is a technology that allows for identification of surface proteins and receptors and thus allows an estimation of cell properties and type.

I was also involved in the optimization and troubleshooting of the NanoPro 1000 system, developed by ProteinSimple, which is an automated Western Blotting system. Western Blots are used to identify the amount of protein in each sample, which allows researchers to identify and quantify the levels of proteins involved in the disease process, and test whether our therapeutic interventions affect these levels. This technology has multiple advantages over traditional protein quantification assays. Firstly, it allows sensitive quantification of protein levels from as little as 25 cells per assay (nanolitres of sample). Traditionally, we would use anywhere from 5,000 to 100,000 cells to be able to get accurate reads. The NanoPro achieves this level of high fidelity by being a charge-based assay – proteins are lined up according to standards based on their charge in a nano-sized capillary, and then tagged with a fluorescent dye. The brightness of a specific peak then corresponds to the protein levels of a particular protein. Secondly, the NanoPro allows additional information rather than just levels of specific proteins. Proteins in the cell can undergo modification procedures, for example the addition of a phosphate group – this process is termed phosphorylation. Because the NanoPro is so sensitive, its able to detect these miniscule changes to the proteins, something that can be done on Western Blots but not without

great difficulty and accuracy – potentially compromising the integrity of these results. Third, the NanoPro allows the processing of 96 data points per go, with a turnover time of about 4-6 hours, depending on the protein being quantified. To put this into perspective, Western Blots can take anywhere from 6 to 48 hours, and with far fewer samples run during that time. During my time with Prof Tan's lab, we were able to advance our troubleshooting steps for a particular protein that could hold key importance in perinatal brain injury. We are now hoping to write up these results into a technical paper to share our methodology with the public.

Finally, the experience and vast knowledge of the team, as well as the equipment available at this institution, truly opened my eyes to the possibilities back home. The technologies available for use were astounding, for example one particular machine had the ability to cut down what would take 3 to 4 days in our laboratory in Melbourne to just a 6 hour process, with greater fidelity and sensitivity. Whilst the funding situation in Australia may not support the purchase of all the equipment that Prof Tan's laboratory had for their own private use, I can certainly foresee this happening in the future – particular with the knowledge and insight I have gained from my time there.

OTHER FINDINGS

Whilst in the United States and Canada, I had the opportunity to also visit other world-leading laboratories in the maternal and perinatal research fields. As a Research Fellow associated with the Amnion Cell Biology, Fetal and Neonatal Health, as well as the Maternal and Perinatal Medicine groups, I manage multiple projects as well as several students in both the Bachelor of Biomedical Science (Hons.) and Bachelor of Medical Science and Doctor of Medicine (MD) courses at Monash University Australia. One other project involves pre-eclampsia, and for this I also visited the laboratories at the Lunenfeld-Tanenbaum Research Institute at the Mount Sinai Health System.

NOVEL THERAPIES IN PRE-ECLAMPSIA

LUNENFELD-TANENBAUM RESEARCH INSTITUTE, TORONTO

Preeclampsia is a serious systemic disorder unique to human pregnancy. About 5% of pregnancies are affected by preeclampsia – equivalent to about 15,000 women a year in Australia. Preeclampsia is a leading cause of maternal and perinatal morbidity and mortality worldwide. It is the leading cause of iatrogenic preterm birth in Australia. The diagnosis and best management of preeclampsia remain a challenge. While it presents as high blood pressure, often with ankle swelling, preeclampsia is essentially due to widespread maternal endothelial dyfunction, which, if unchecked, can lead to liver and renal failure, stroke and pulmonary oedema. It is only "treated" by delivery of the baby and placenta. The underlying cause of the endothelial dysfunction is thought to be the release of anti-angiogenic and vasoactive substances from a damaged placenta into the maternal circulation. These compounds, including soluble fms-like tyrosine kinase 1 (sFlt-1), and soluble endoglin (sEng), then cause downstream maternal inflammation and oxidative stress, and results in the manifestation of the clinical symptoms.

Visiting the laboratories of Dr Stephen Lye located at the Mount Sinai Health System in Toronto; I managed to secure a meeting with Dr Caroline Dunk who is a Research Associate there. Dr Dunk shared her research findings on her extravillous trophoblast (EVT) explant work, crucial to understanding the mechanisms behind EVT invasion during pregnancy, and

what happens when this well regulated process is disrupted in disorders of pregnancy. It is well known that failure of the spiral arteries of the uterus to remodel correctly, as well as improper trophoblast invasion, result in the pathophysiologies seen in pre-eclampsia and for example fetal growth restriction. Isolating these cells and performing experiments in culture allow important determination of pathways that are disordered in these diseases, and how we can develop new therapies for them. As a result of my visit, we opened up potential collaborative avenues and I certainly hope to keep the conversation between both our laboratories ongoing.

Our laboratory is actively researching new and novel compounds that can target both the inflammatory and oxidative stress stimuli, in an attempt to arrest the hypertension and endothelial dysfunction. Our focus has been on identifying pathways involved in the disease process, and either developing or testing new pharmaceutical or naturally occurring antioxidant therapies that can be isolated from fruits or plants. However, to assess these therapies in the robust manner required for translational research and to progress these findings towards future clinical trials, a biologically relevant and accurate animal model of disease is required. We have so far attempted to replicate a rat model of pre-eclampsia, which develops clinical symptoms of the disease by viral transfection, causing up-regulation of sFlt-1 and sEng – the proteins responsible for the clinical symptoms during pregnancy. We combine this with radiotelemetry, which is increasingly being recognized not just as the gold standard, but a necessity for validation of gestational hypertension seen in preeclampsia.

Radiotelemetry is a high fidelity state-of-the-art method [6, 7] which allows real-time recording of arterial pressure, venous pressure, kidney pressure, ECG, EMG, EEG, glucose, temperature, activity and numerous other parameters in small or large animals [8]. Current technology is now sufficiently developed to allow high fidelity blood pressure recordings over several months with little or no drift. This is of particular interest for preeclampsia research, as real-time recording of blood pressure over gestation would allow better elucidation of novel therapies that can both target the underlying endothelial dysfunction, but also manage the clinical hypertension.

Serendipitously during my trip I was introduced to John Kingdom's laboratory, where I met Jovian Wat, a PhD student who is currently working on models of pre-eclampsia. This particular animal model utilised rats, and was first established in the Granger lab at the University of Mississippi. The particular surgery technique is quite well known in pre-eclampsia research and has been validated by several other groups around the world. The model is based on the finding that reduced uteroplacental blood flow causes defects in angiogenesis and invasion crucial for the clinical symptoms of pre-eclampsia. To summarise the surgery briefly, clips are placed on both sides of the ovarian vessels for a short period of time, thereby reducing bloodflow and causing disease. In my continued discussions with this group it became evident that both our groups had invested substantial amounts of time and resources into models that could not replicate and reproduce consistent symptoms of pre-eclampsia. Having established this I was able to get a key contact at a collaborator's laboratory that would be able to train me in this surgery. This could potentially save our laboratory months in training and valuable resources, as well as cements the relationship between both world-leading groups.

CONCLUSIONS AND RECOMMENDATIONS

This was a life-changing undertaking for me as an early career researcher. Given the success of medical research in the United States in translating research findings towards actual therapies in the clinic, I was keen to learn from a leading cerebral palsy research laboratory and indeed – I learnt and grew a great deal while I was there. Seeking to establish a model of hypoxia-induced cerebral palsy in rabbits, I outline below key learning experiences from my travels:

- This model, already highly validated in the research field, fulfills a missing niche in our research center back at the Hudson Institute. The model recapitulates accurately the motor symptoms seen in human CP, which provides us a powerful tool to assess future human therapies and potentially progress them to clinical trials;
- Establishing intercontinental bridges for collaboration is essential to success. I have learnt so much in such a short amount of time, not just with scientific skills but professional skills as well. I hope to begin conversations to establish a visiting fellowship for the postdoctoral fellows there to come to Melbourne and help us

establish a world-leading cerebral palsy research platform – and animal models are the foundation of this;

- Infrastructural and administrative changes to improve the efficiency of our research. Despite the advantage of having superior access to cutting-edge equipment in the United States, I have learnt a great deal on optimizing our facility layouts as well as equipment that will confer significant savings to our current setup at the Hudson. I hope to begin discussions with our animal and platform facility managers on whether these can be implemented here, as based on my experience in Detroit. These include improvements in software used to manage our animal tracking, better analysis software, surgical equipment that will save both time and money;
- On the pre-eclampsia research front, my exposure to the model being used at the Mount Sinai Hospital has given me great confidence that this is the one we should be pursuing as our groups' preferred pre-eclampsia model. Given the high reproducibility of the clinical symptoms as well as ease of surgery and post-operative care, I hope to now begin discussions with my supervisor to begin collaborations with the lab who pioneered this surgical technique, and will allow us to begin assessing new therapies for pre-eclampsia in earnest.

There are several ways I hope to disseminate these findings, but in my position as an academic researcher I am fortunate to have access to channels for this purpose. The first and most obvious is via journal publications. During my time in Detroit I generated sufficient data from the three projects I was involved in to potentially merit authorship on those papers. Two of these projects involved understanding the pathways leading to cerebral palsy, and results have already been generated for the publication of this data. We hope that these papers will present novel pathways, not yet investigated, in the genesis of cerebral palsy, and thus stir conversation and discussion as potential therapeutic targets. I also assisted on a technical paper optimizing the NanoPro system, as discussed above. We went through a significant amount of troubleshooting and optimization and hope to have this technical paper written up and published by the end of the year. Besides these papers, I now aim to begin the process of starting up this animal model here in Australia. My learnings from my time in Detroit will be disseminated to our facility managers, group leaders and my supervisors either through

departmental seminar, or private meetings. Besides this, the findings from my projects will also be shared at conferences and seminars, most likely in perinatal or cerebral palsy research, either by Prof Tan or from members of his laboratory. It is my hope that once this model is up and running in Melbourne I will then also be able to share my experiences in starting up this model here, as well as train potential collaborators who would like access to it.

The changes required to bring this model to life are not simple. I will first begin conversations with the facility managers to purchase the equipment needed for the surgeries, but also begin negotiations for the space required to both house and care for rabbits. Currently, we do not have any rabbit models of disease at our centre, so educating the facility technicians and carers on how best to care for these animals, or if required, hiring technicians who have this experience already, will be needed. I was also fortunate enough to have access to proposals and equipment requests from Prof Tan's lab, which will hopefully assist me in getting the funding required for these changes. I will begin applying to both philanthropic and governing bodies for the research money needed for this project, using the knowledge I have gained over at Wayne State University.

Currently, we have several models of perinatal brain injury that could be used for cerebral palsy research at our centre. However, none of these models recapitulate the motor symptoms as accurately as the rabbit model of hypoxic injury. The accuracy of the disease model is vital for the translation of the research to the clinic. I hope to now involve other neuroscientists and cerebral palsy researchers to collaborate and begin talks to make our experimental therapies a reality.

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