2020 RESEARCH REVIEW

Research For Life

WELLINGTON MEDICAL RESEARCH FOUNDATION

RESEARCH REVIEW 2020

COVER PHOTO:

Dr Abigail Sharrock received a Research For Life grant to undertake research into the improvement of gene delivery vectors for gene directed enzyme prodrug therapy (GDEPT). GDEPT is a targeted anticancer strategy being developed to address the limitations of current chemotherapies including unwanted associated side-effects due to low selectivity of chemotherapeutic drugs. Abigail is a Postdoctoral Fellow in Biotechnology in Professor David Ackerley's Microbial Biotechnology Research Group at Victoria University of Wellington.

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Research For Life
The Wellington Medical Research Foundation Incorporated

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Editorial

BY ASSOCIATE PROFESSOR REBECCA GRAINGER

On writing editorials that end a year, one generally reflects on the events that have passed.

The global COVID-19 pandemic is a very significant event in our collective history, both in Aotearoa New Zealand and internationally. Although public health experts have been warning for years that a globally significant pandemic was a very real threat, we probably had a collective "not us, not here" response. Although New Zealand has fared well with controlling the number of cases of COVID-19 and subsequently deaths and morbidity, the economic, social and personal impacts are enormous and will be ongoing. We should all acknowledge that good science and good science communication have been the backbone of New Zealand's success in controlling COVID-19.

Science is fundamentally about asking questions and figuring out appropriate ways to find the answer. For the most part, science is incremental; big breakthroughs are few and far between and most findings only subtly shift understandings. Often, more new questions are raised than were answered. At the start of the pandemic we extrapolated knowledge about infection transmission and biological plausibility of treatments from other respiratory viruses to the SARS CoV-2 virus, that cause COVID-19. This was useful for some aspects such as mask wearing and transmission but less successful, even damaging, for other aspects. One such example was the hype around use of hydroxychloroquine to treat COVID-19, resulting from promotion of preliminary studies which were very poor quality. This caused significant harm to people with rheumatic disease who were suddenly unable to access their long-term medication. The scientific community internationally has risen to the challenge by vocally challenging promotion of poor science and pivoting to undertake high quality COVID-19-related research. This research covers the breadth of science from basic virology to public health and everything in between. There has been an unprecedented level of collaboration, activity and publishing on COVID-19 in 2020. All these scientists have years of training behind them starting at universities decades ago. We are fortunate that these people have invested this time in developing skills, knowledge, and the networks and teams to undertake this vital research.

We should also celebrate local science leaders who are contributing on a national and international level. Never would I have thought a Wellington academic in public health would become a household name but Professor Micheal Baker has achieved just that. Professor Baker, Professor Tony Blakley, and his colleagues at University of Otago Wellington have made enormous contributions to the science-based approach of government policy. Dr Ayesha Verrall brought her scientific skills in infectious disease and epidemiology to bear providing essential feedback to firm up and improve the contact tracing approaches that control new community infections. The Malaghan Institute of Medical Research (MIMR) is now focusing on COVID-19 vaccine research in collaboration* with Victoria University of Wellington in the Vaccine Alliance Aotearoa New Zealand – Ohu Kaupare Huaketo. Research For Life has had a long history of supporting scientists, particularly student research, at the MIMR so it is with particular pride that we can recognise the contribution of the team at MIMR under the leadership of Professor Graham le Gros. It has never been more important to support the Wellington scientific research community in their research, and especially in the role of developing the scientists of the future.

While the scientific community has risen to address the big questions of COVID-19, there have also been challenges. Community alert levels have made laboratory and field work more challenging, and probably more lonely when essential work continued under Level 4 with non-essential colleagues at home. While alert levels in Wellington have remained below Level 3, scientists are planning for ways that science can continue if that changes. Finally, there are now no practical opportunities to engage in person with international collaborators. We are all making the best of online or virtual conferences to disseminate important findings and exchange ideas. Overall, this is effective if you can remain alert in the middle of the night when "zooming" in to international meetings!

As a member and now Chair of the Research Advisory Committee I have had the pleasure of reading many grant reports from our scientists in Wellington. These are our best and brightest, who are finding creative and cutting edge ways of addressing important health problems from right here in Wellington. Research For Life is committed to continuing to support the development of science in Wellington by supporting their research, and particularly that of our younger and emerging talent. We never know when we will need them.

*https://www.malaghan.org.nz/infectious-diseases/covid-19/

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RESEARCH REPORTS

University of Otago, Wellington

Measuring extracellular matrix remodeling and myocardial repair during the acute phase of myocardial infarction

M BRUNTON-O'SULLIVAN, A HOLLEY, S HARDING, P LARSEN

KEY FINDINGS

- A majority of extracellular matrix (ECM) biomarkers measured in this study were shown to have altered expression levels in acute myocardial infarction (AMI is more commonly known as a heart attack) patients compared to healthy volunteers.
- ECM biomarkers were temporally dynamic in patients following AMI.
- The variation observed in ECM biomarkers post-MI was only modestly described by parameters of patient health, suggesting change in these biomarkers is driven by factors independent of clinical characteristics and heart function.

OBJECTIVES

The cardiac ECM provides structural support and organisation to the heart and is radically altered following a heart attack. Termed ECM remodeling, damaged tissue is replaced by non-contractile scar and is a process tightly regulated by multiple cellular and molecular factors.

Inadequate ECM repair following a heart attack can predispose a patient to increased heart dysfunction and adverse cardiovascular outcomes. Measuring ECM biomarker dynamics in the acute phase of AMI, and identifying what drives changes in biomarker levels, is an important initial step towards identifying potential biomarker targets to risk-stratify patients.

Therefore, the primary objective of this study was to use a panel of ECM biomarkers to measure the magnitude and time course of the ECM response following a heart attack. This was achieved by investigating the following aims: 1) identifying differences which exist in ECM biomarker levels between MI patients and healthy volunteers, 2) examining temporal changes in ECM biomarkers during acute presentation (Day 1 - 3) and at a chronic six-month measurement, and 3) identifying which clinical characteristics and parameters of cardiac function drive changes in ECM biomarker levels of MI patients.

METHODS

PATIENT RECRUITMENT. We prospectively recruited 23 patients presenting to Wellington Regional Hospital with AMI. All patients were recruited within 24 hours of symptom onset. Blood samples were collected on Day 1, Day 2, Day 3 and at six months following symptom onset. Blood samples from 12 age-matched volunteers were included as a reference control for this study. To be included as a healthy volunteer, participants were required to have no known cardiovascular disease or other acute illness in the preceding six weeks to blood collection.

BIOMARKER MEASUREMENT. A combination of ELISA and Human Magnetic Luminx® multiplexing assays were used to measure the levels of all extracellular matrix (ECM) biomarkers. These measurements included: matrix metalloproteinase-2 (MMP-2), MMP-3, MMP-8, MMP-9, osteopontin, periostin, procollagen I N-terminal propeptide (PINP), transforming growth factor beta-1 (TGF-β1), tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) and TIMP-4.

RESULTS

1) **Most ECM biomarkers demonstrated significantly altered levels in AMI patients compared to healthy controls.** Of the nine ECM biomarkers measured, we identified six which had an altered distribution in the AMI population compared to healthy volunteers **(Table 1)**. Four ECM biomarkers had increased levels either at a single time point (MMP-2) or across multiple time points (MMP-9, PINP, TIMP-1).

   In comparison, both MMP-3 and periostin displayed suppressed biomarker levels across acute measurements, with levels returning to within the healthy reference range at six months. Of the six biomarkers with altered levels in AMI patients, five had changes which occurred at acute time point measurements.

2) **ECM biomarkers with altered expression levels in AMI patients were also shown to be temporally dynamic.** When levels of ECM biomarkers were compared across time points in AMI patients, MMP-2, MMP-3 and periostin had significantly elevated levels at six months compared to acute levels. In comparison, MMP-9 and PINP levels peaked during the acute phase and decreased at chronic measurements. TIMP-1 displayed no changes in biomarker levels across time **(Figure 1)**.

3) **The levels of variation observed in ECM biomarkers were not well described by clinical characteristics or cardiac function.** Age was significantly correlated with levels of MMP-2 at acute and six-month time points (rs=0.579 and rs=0.605, all p<0.01). BMI was weakly and inversely associated with six-month measurements of MMP-2 and MMP-3 (-0.472 and -0.432, all p<0.05).

   Female patients had higher levels of MMP-3 at acute and six-months measurements when compared to male patients (all, p<0.05). Current smokers had lower MMP-2 levels at acute (p<0.05) and six-month measurements (p<0.01), as well as reduced levels of periostin at six months (p<0.01) when compared to non-smoking patients. Area under the curve (AUC) high sensitivity-Troponin T (hs-TnT), a surrogate measurement of heart attack size, was only modestly associated with MMP-3 levels at acute and six-month measurements (0.445 and 0.555, all p<0.05). Left ventricular ejection fraction (LVEF), which is a central measure of heart function, was inversely associated with six-month measurements of MMP-2 (-0.466, P<0.05) and acute and six-month levels of periostin (-0.627 and -0.571, all p<0.01). MMP-9, PINP and TIMP-1 were not associated with any parameters of patient health.

CONCLUSION

In this study, we have identified a group of six ECM biomarkers which have altered levels in AMI patients compared to healthy controls. Furthermore, these biomarkers demonstrate temporal variation between acute time points and six-month measurements. We have shown that changes in these ECM biomarkers are only modestly driven by parameters of patient health, which suggests additional processes are driving biomarker variability. Examining how these ECM biomarkers are associated with disease progression in AMI patients is the next phase of this research.

		Serial Measurements			
Biomarker	Healthy	Day 1	Day 2	Day 3	Month 6
MMP-2	149.60 [144.10-160.20]	166.80 [151.0-184.0]	172.9 [144.70-197.20]	165.90 [152.00-174.20]	**217.30 [181.40-235.80] ******
MMP-3	20.34 [10.32-38.21]	**8.98 [6.64-11.99] *****	**9.71 [8.11-13.95] ***	**9.31 [6.42-14.44] ****	12.78 [9.49-14.86]
MMP-9	17.72 [14.19-31.43]	**58.60 [43.00-94.21] ******	**63.83 [49.13-101.20] ******	**56.16 [39.02-69.27] *****	36.43 [28.53-50.39]
Osteopontin	36.70 [13.37-56.10]	52.09 [40.72-70.89]	57.03 [41.69-66.00]	55.97 [36.79-80.20]	41.00 [30.46-47.32]
Periostin	115.90 [89.22-133.6]	**71.60 [60.74-94.75] ****	**75.79 [66.39-89.92] ****	**72.42 [60.78-90.24] ****	88.54 [64.98-112.90]
PINP	60.31 [54.27-73.09]	**81.32 [69.89-93.68] ***	**82.31 [73.39-99.00] ***	**90.72 [79.03-100.00] ****	81.94 [71.22-90.31]
TGF-β1	2.10 [1.97-3.84]	2.21 [1.65-4.02]	2.23 [1.64-2.83]	2.42 [2.05-3.06]	2.47 [2.17-3.51]
TIMP-1	60.33 [52.27-70.64]	**75.04 [67.81-83.97] ***	**80.78 [64.47-101.00] ****	**78.08 [66.47-93.50] ****	**77.08 [64.53-87.08] ***
TIMP-4	3.05 [2.62-3.92]	4.10 [3.20-5.19]	3.62 [2.21-4.86]	2.98 [2.30-3.59]	3.58 [2.74-]

Concentrations are expressed as median [IQR] ng/mL. Kruskal-Wallis multiple comparisons test with Dunn's correction was performed to identify differences between healthy and longitudinal MI biomarker concentrations. * indicates significant differences. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

TABLE 1: Extracellular matrix biomarkers levels in AMI patients and healthy volunteers.

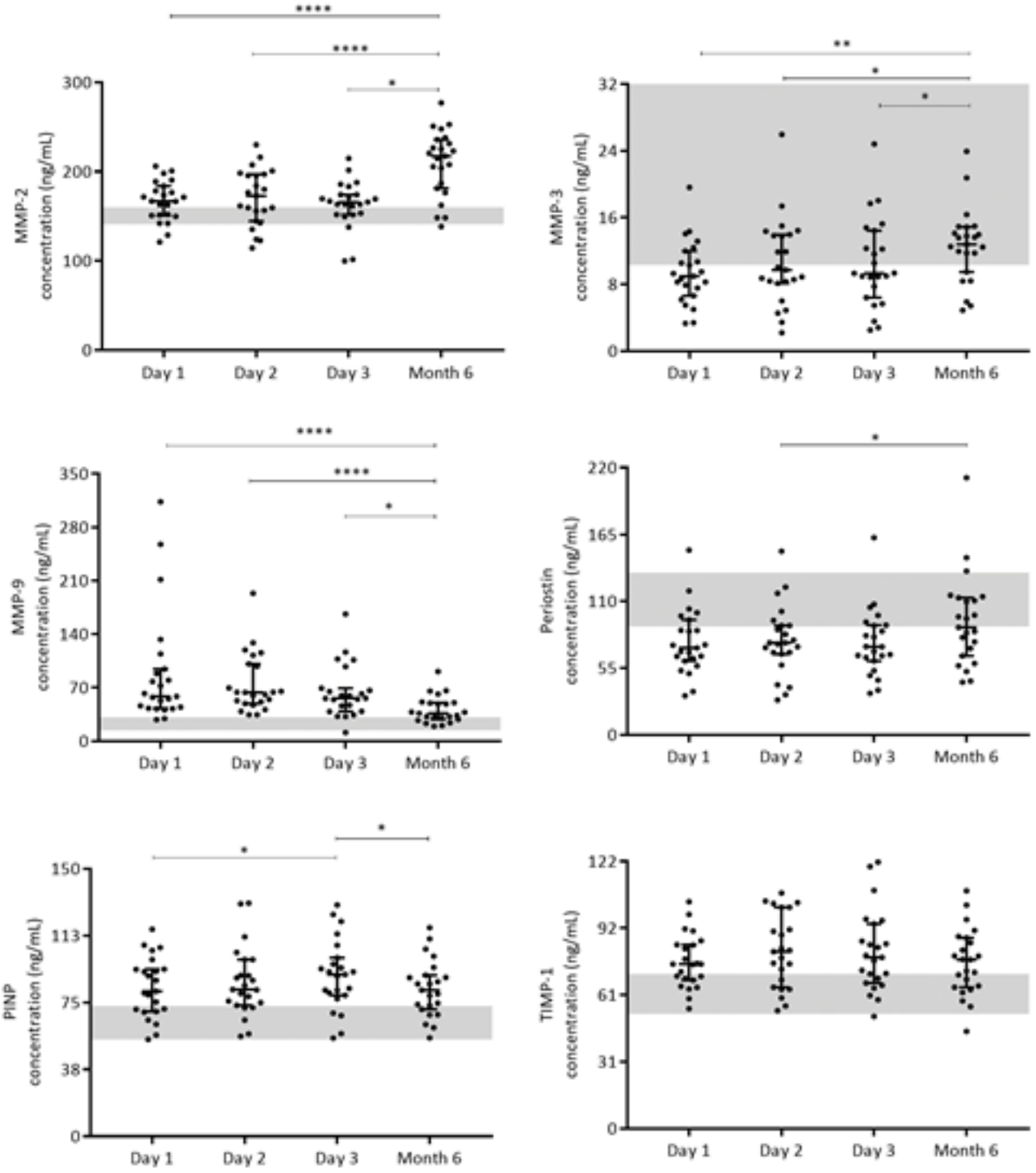


FIGURE 1. The temporal profile of ECM biomarkers in AMI patients. Median (IQR) of ECM biomarkers is shown. Grey shaded area is the IQR of healthy controls. Friedman multiple comparisons test with Dunn's correction was performed to identify differences between biomarker levels across time. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

University of Otago, Wellington

Extracellular vesicle mircroRNA as liquid biopsy biomarkers in early stage colorectal cancer

B DESMOND, E DENNETT, K DANIELSON

KEY FINDINGS

Funding provided by this Research for Life Grant was used for the purchase of lab materials to evaluate microRNA (miRNA) dysregulation in both colorectal cancer (CRC) tissue and plasma extracellular vesicles (EVs) from stage I and II CRC patients.

Four miRNA, miR-19a, miR-23a, miR-1246 and miR-23a were examined to assess their suitability as liquid biopsy biomarkers. All had shown promise as possible biomarkers in CRC in the literature. Two miRNA, miR-1246 and miR-183, were significantly overexpressed in CRC tissue compared to matched normal colonic mucosa. miR-19a was unchanged in tumour tissue but was significantly downregulated in the EVs of early stage CRC patients compared to healthy controls. miR-19a may have potential as a blood based, liquid biopsy biomarker.

OBJECTIVES

The objectives of this study were to establish whether the candidate miRNA could be used to differentiate early stage CRC from controls in both tissue and plasma EVs. This was in a well-defined cohort of stage I and II CRC patients with matched CRC tumour tissue and normal colonic mucosa. Plasma samples included all patients where tissue was examined in addition to further stage I and II patients for whom only plasma was available. Control plasma EVs were sourced from a cohort of patients who had an entirely normal colonoscopy to rule out any polyps or other colonic abnormalities that may have had an effect on expression of the candidate miRNAs.

If these microRNAs are differentially expressed in tissue and in circulating EVs then they may have utility as liquid biopsy biomarkers.

RESULTS

Funding from the grant was used to purchase materials to isolate EVs from plasma, extract RNA from both tissue and EVs and subsequently to perform Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) to quantify miRNA expression.

In tissue, forty-two patients with matched tumour tissue and normal colonic mucosa were examined. miR-183 had a 210-fold increase in expression **(Fig 1 A, p = 0.0008)** and miR-1246 had a 148-fold increase **(Fig. 1 B, p = 0.0004)**. Plasma EVs were extracted from sixty-six early stage CRC patients in addition to thirty healthy controls. Changes in miR-183 and miR-1246 in tissue did not translate to the plasma EVs and neither of these miRNAs were consistently expressed in the circulation when using our methods. However, miR-19a, which did not show any change in tissue, demonstrated significantly decreased expression in plasma EVs of CRC patients when compared to that of healthy controls. This corresponded with a 0.218-fold decrease in expression **(Fig 1 C, p = 0.0043)**. The ability of plasma EV mir-19a was evaluated using Receiver Operating Characteristic (ROC) curves, this corresponded with an Area Under the Curve (AUC) of 0.6104 **(Fig 1 D)**. This was compared to Carcinoembryonic Antigen (CEA) a known blood-based marker in CRC, which demonstrated an AUC of 0.6576 in our cohort. CEA is not used as a diagnostic biomarker due to limited sensitivity and specificity in early stage CRC. When miR-19a and CRC were combined, their ability to differentiate healthy patients from CRC was not additive.

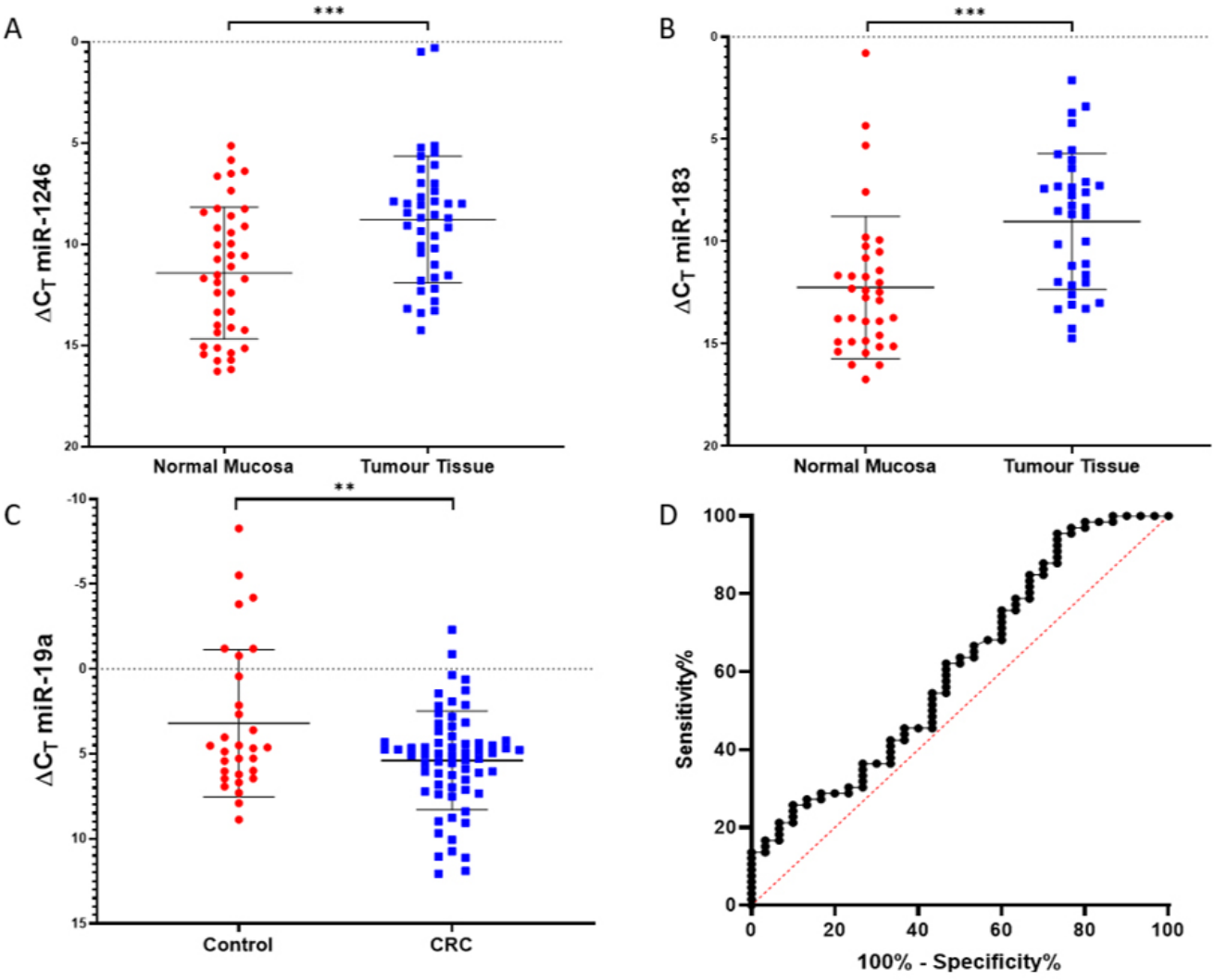


FIGURE 1:

(A) miR-1246 CRC tumour tissue vs normal colonic mucosa ($p = 0.0004$)

(B) miR-183 CRC tumour tissue vs normal colonic mucosa ($p = 0.0008$)

(C) miR-19a plasma EVs CRC vs controls ($p = 0.0043$)

(D) ROC curve miR-19a (AUC 0.6104)

CONCLUSIONS

Four candidate miRNA were evaluated for their use as liquid biopsy biomarkers in early stage colorectal cancer. If an effective liquid biopsy was developed it would likely both improve both patient centered outcomes and overall healthcare costs for CRC. One of these miRNA, miR-19a, has shown to be differentially expressed in the circulation of CRC patients. It appears that this is the first time a decrease in this marker in CRC patients has been observed and it warrants further assessment as to its suitability as a liquid biopsy.

AWARDS AND PRESENTATIONS

This research was awarded the prestigious Louis Barnett Prize for 2019 by the Royal Australasian College of Surgeons when presented at the RACS Surgery 2019 conference. It was presented at the New Zealand Society for Oncology annual conference in 2019 and poster presentations were also made at two international conferences: the European Society for Coloproctology ASM 2019 in Vienna and the Australasian Gastrointestinal Cancer Trials Group ASM 2019 in Adelaide.

University of Otago, Wellington

Ambient temperature, appetite and food choices

T O'DONNELL, J KREBS, J MILES-CHAN

KEY FINDINGS

This research investigated the effect of different temperatures on energy expenditure and the desire to eat and the type of foods eaten under warm and cold environments. We found that whilst exposure to cooler temperatures increased the amount of energy expended, it did not influence the amount or type of food consumed.

AIM

This research sought to test the hypothesis that exposure to cooler ambient temperatures will increase energy expenditure and that this will be matched by an increase in consumption of food.

METHODS

This study consisted of 12 male volunteers between the ages of 18-35 with a BMI of less than 30. All participants were healthy and had sedentary professions. The study was described to prospective participants as an investigation researching how temperature may influence productivity and concentration in an office environment. This deception was required to avoid conscious food selection.

Participants made three visits to the University of Otago after fasting for 12 hours. After changing into standard hospital scrubs, participants spent two hours inside an environmental simulation suite under three different temperature conditions: cold (13°C), normal (23°C) and warm (33°C). Humidity was kept at a constant 40% for each temperature. The first 25 minutes was spent on a computer-based test performed whilst sitting at a desk.

Energy expenditure was then measured using indirect hood calorimetry. This test requires participants to remain still for 30 minutes while the participants expired breath is collected. Using calculations based on the volume and ratio of carbon dioxide and oxygen in the breath, accurate measures of resting energy expenditure can be made.

On completion of the calorimetry recording, participants were then asked to remain in the environmental simulation suite for a further 60 minutes for the purpose of collecting questionnaires and other physiological data. During this time a wide and large volume of food was provided, and participants were encouraged to eat as much as they desired. The selection of food included a range of healthy foods such as fruit, and unhealthy snacks such as donuts. Food volume was weighed before and after the experiment.

Any differences between groups for food consumption and resting energy expenditure measures were assessed with a repeated measures of one-way ANOVA analysis. Statistical significance was set at $p<0.05$.

RESULTS

Figure 1 shows that energy expenditure during the cold exposure session was significantly higher ($p = 0.001$) than during either the normal or warm conditions. There was no significant difference between the normal and warm conditions.

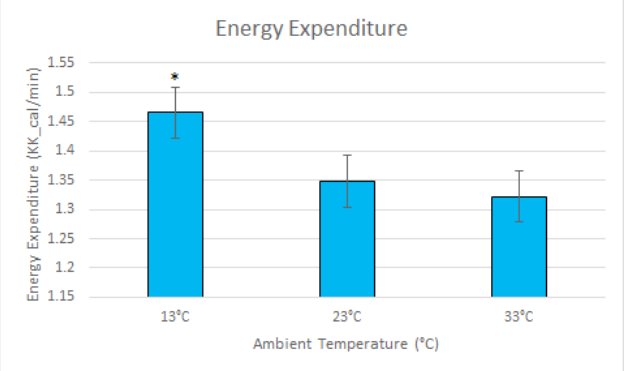


FIGURE 1: Resting energy expenditure measured under cold, normal and warm temperature conditions. *$p < 0.05$.

Table 1 shows the average food volumes and compositions consumed by the participants during the 60 minutes of each experimental condition. There were no significant differences between the experimental conditions suggesting that temperature did not influence appetite or the drive to eat. Under all condition's participants consumed approximately 50% of their recommended daily intake within the 60 minutes provided.

CONCLUSION

These results demonstrate that while a cooler ambient temperature leads to an increased energy expenditure, this is not balanced out in the short-term by an increased drive to eat.

Our previous research reported on a range of temperatures that were able to increase energy expenditure without causing participant discomfort. This suggested the potential for ambient temperature interventions as an obesity prevention tool. However, several participants described feeling particularly hungry following exposure to cooler temperatures. It was hypothesised that any increase in energy expenditure caused by the cooler temperatures may be counterbalanced by an equal increase in food consumption.

Therefore, this study sought to examine the physiological drive to consume food during cooler conditions. These results reinforced the hypothesis that cooler temperatures increase the energy expended. However, the hypothesis that this may be matched by an increase in food consumption was not supported.

While this research focused on timeframes and temperatures that may be experienced in an office environment, further research into more extreme temperatures, such as reported in older NZ homes, and for longer exposure periods, would be instructive to validate these findings. Based on these results, cold exposure shows promise as an obesity intervention given the potential to increase energy expenditure without impacting food consumption.

	Cold 13°C	Normal 23°C	Warm 33°C
Food weight consumed (g)	780.9 ± 237.8	743.6 ± 252.1	748.3 ± 235.1
Energy consumed (kJ)	5637.9 ± 1081.9	5456.5 ± 1413.5	4908.1 ± 1816.4
Carbohydrates (g)	224.2 ± 55.4	220.2 ± 65.9	204.9 ± 75.4
Fat (g)	37.3 ± 10.8	34 ± 13.4	32 ± 14.9
Protein (g)	34.9 ± 8.4	32.8 ± 9.3	29.6 ± 10.3

TABLE 1: Food consumption under different temperature conditions; mean ± standard deviation.

University of Otago, Wellington

A randomised controlled trial of additional bolus insulin using an insulin-to-protein ratio compared with insulin-to-carbohydrate ratio alone in people with type 1 diabetes following a carbohydrate-restricted diet

H WRIGHT, A PARRY-STRONG, B CORLEY, R HALL, J KREBS

KEY FINDINGS

Some people with type 1 diabetes who follow a low carbohydrate diet find it helpful to give extra insulin for the protein they eat. A guide ratio commonly used for this is to halve the insulin to carbohydrate ratio. Trialing this in a group of 34 people found that diabetes control was not better using the ratio compared to not giving extra insulin for protein. While some people still found it helpful, others found it stressful.

OBJECTIVE

The addition of bolus insulin for meal protein content may be helpful in controlling post-prandial glycaemia in people with type 1 diabetes mellitus (T1DM) following a carbohydrate-restricted diet. This study evaluated the effect of an insulin-to-protein ratio in addition to an insulin-to-carbohydrate ratio over 12 weeks in people with T1DM following a carbohydrate-restricted diet.

METHODS

Participants with T1DM aged ≥18-years who used a basal-bolus insulin regimen or a subcutaneous insulin pump were randomly allocated (1:1) to either carbohydrate and protein-based insulin dosing or carbohydrate-based insulin dosing alone for 12 weeks. All participants followed a carbohydrate-restricted diet (50-100g per day). Measurement of HbA1c (primary outcome), weight, height and blood pressure occurred at baseline and 12 weeks. Participants used two-week food diaries and continuous glucose monitors prior to these visits and completed a questionnaire about their experience at the last visit.

RESULTS

Thirty-four participants were recruited, 16 to the intervention group and 18 to the control group. One participant withdrew from the intervention group and two from the control group. There was a small non-significant improvement in HbA1c in both groups after 12 weeks, adjusted for baseline HbA1c, with a mean (SD) in control group of 55.7 (10.6) mmol/mol and intervention group 52.3 (7.2) at 12 weeks (p=0.65). Using CGM data, the intervention did not significantly improve glycaemic variability, time spent in normal glycaemic range or hyperglycaemia compared with the control. There was no significant increase in hypoglycaemia and no significant changes in weight or blood pressure for either group. While some participants found the insulin-to-protein ratio helped them feel in control of their diabetes, others noticed a higher level of distress.

CONCLUSION

Additional bolus insulin using an insulin-to-protein ratio did not improve glycaemic control in patients with T1DM who follow a carbohydrate-restricted diet. The risk of hypoglycaemia did not increase.

Malaghan Institute of Medical Research

High dimensional spectral flowcytometric characterisation of intestinal immune cell populations during helminth infection

L FERRER-FONT, P MEHTA, P HARMOS, AJ SCHMIDT, S CHAPPELL, KM PRICE, IF HERMANS, F RONCHESE, G LE GROS, JU MAYER

KEY FINDINGS

We were able to establish a refined cell isolation protocol that allowed us to extract millions of immune cells from parasite infected intestines. We further characterised these cells using a novel technology called 'spectral flow cytometry' and identified several waves of immune cell infiltration throughout the course of infection.

OBJECTIVES

In our original research proposal, we had identified 3 main objectives.

- Establish and optimise a digestion protocol for helminth infected intestines.
- Develop a high-dimensional 24-color flow cytometry panel to assess tissue specific type 2 immune responses in the intestine.
- Characterise anti-helminth type 2 immune responses in the infected intestine and analyse differences in the immune cell repertoire at different stages of infection.

We were able to meet all three objectives in the proposed time frame and recently published our findings in an international peer-reviewed journal (Ferrer-Font et al., High-dimensional analysis of intestinal immune cells during helminth infection, eLIFE, 2020; DOI: 10.7554/eLife.51678, https://elifesciences.org/articles/51678).

METHODS

The standard procedure to obtain immune cells from the gut consists of three steps: collecting a gut segment and washing it, stripping away the surface layers with chemicals, and finally using enzymes to digest the tissues, which are then filtered to obtain individual cells.

However, cell isolation from parasite infected intestines has been notoriously difficult, as the infection creates strong local responses — such as an intense 'slime' production — to try to flush out the worms.

In order to overcome this significant obstacle and obtain cells for our analysis, we methodically refined each step of the protocol and tested over 15 different variations before finally succeeding to collect millions of immune cells from infected intestines.

In parallel, we developed a large antibody panel that would allow us to identify a variety of different immune cells that could be involved in anti-parasite immunity. We decided to use a novel technology, called 'spectral flow cytometry' to study these cells, which had recently been installed at our institute. Using this technology, we were able to identify up to 40 different immune cells, whereas a similar approach using 'conventional flow cytometry' would have only allowed us to identify 10 cell types at a time.

Lastly, we developed methods to visualise different immune cells by microscopy, to allow us to investigate where these cells were located within intact tissue sections.

FINDINGS

We were able to isolate and study over 40 different intestinal immune cell types and investigate them throughout the course of a parasite infection. Many of these immune cells could be grouped into different classes. Some immune cells were present even before the infection took place and belonged to cell types that are known to exist in all tissues to monitor the environment and respond to danger. As expected, these cells drastically changed their behaviour upon infection in order to activate and warn other immune cells. A first wave of innate immune cells, so called 'first responders' followed, which were recruited to the intestine early on, when the parasites were still in development. By microscopy we could show that these first responders were indeed found around the developing larvae and were likely attacking them. Once the larvae had developed into adult worms, these first responders disappeared quickly and were replaced by immune cells of the adaptive immune system, which are more specific against individual pathogens but need time to develop. Over time, these cells also decreased suggesting that the infection had been eliminated or that the parasite had found ways to disguise itself from immune detection, as previously shown by other research groups.

CONCLUSION

We were able to develop an optimised isolation protocol to extract millions of immune cells from parasite infected intestines. We paired this new extraction process with 'spectral flow cytometry' to study and characterise these immune cells further and identified several waves of immune responses throughout infection. We anticipate that our cell isolation protocol can also be paired with other technologies that can, for example, reveal which genes are turned on in individual cells. This could help map out exactly how the body fights parasite infections, and how to improve this response. In addition, our protocols could also be useful to extract immune cells from the gut in other challenging scenarios, such as food allergies or inflammatory bowel disorders and allow researchers to understand these conditions in greater detail.

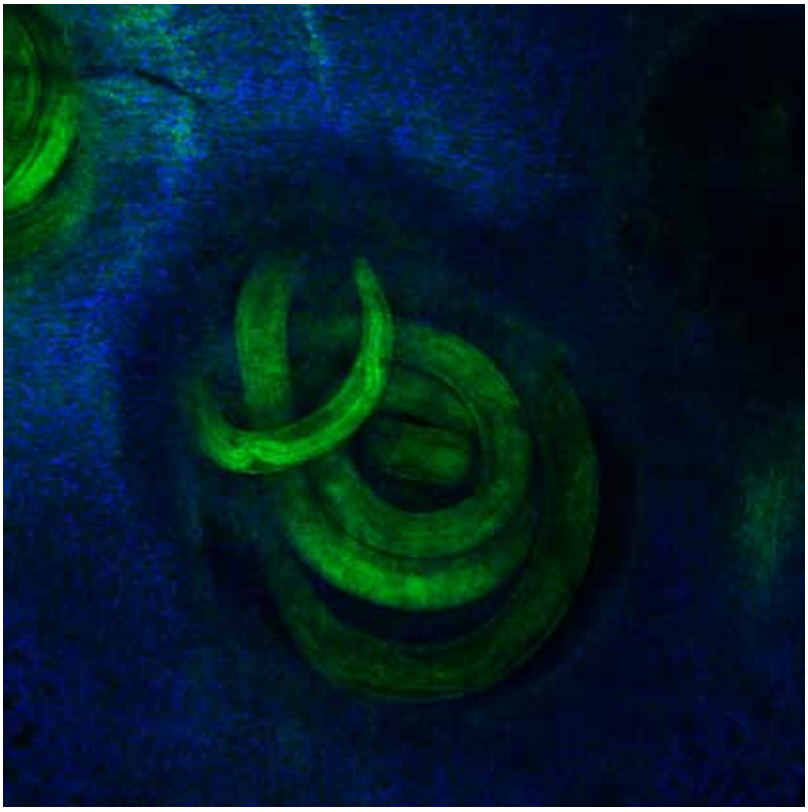


IMAGE: Microscopic image of parasite larvae (green) developing within the intestine (blue)

Victoria University of Wellington

Lethal injection: how a bloodborne bacterial pathogen avoids immune activation

JK MACKICHAN

KEY FINDINGS

Two *Bartonella quintana* effectors, directly injected into host cells by a Type 4 secretion system, were studied here; both inhibit the innate immune system, contributing to long-lasting *B. quintana* infections. Here we showed that one of these effectors, BepA1, inhibits the secretion of multiple pro-inflammatory cytokines. The second effector, YopJ, inhibits cytokine secretion by interfering with innate immune signaling pathways.

OBJECTIVES

Our preliminary studies suggested that both YopJ and BepA1 from *Bartonella quintana* inhibit the expression of the proinflammatory cytokines IL-6 and IL-8 from cells stimulated with TNF-a or LPS. Previous experiments used a yeast two-hybrid approach to identify candidate host cell proteins that are post-translationally modified by YopJ or BepA1. YopJ is a secreted effector with transacetylase activity; the acetylation of host target proteins, especially those involved in signaling, likely impairs their function. This may occur through disruption of other modifications (e.g., phosphorylation) or of interactions with signaling partners. BepA1 has motifs suggesting AMPylation activity, which alters host cell proteins via an inappropriate post-translational modification, likely also interfering with normal host cell signaling and function. Because the critical residues of transacetylase and AMPylation catalytic motifs are well known, we generated catalytically dead variants of both effectors, and these were transfected into host cells alongside the wild type enzymes.

Our aims were (1) to generate isogenic mutants in the YopJ and BepA1 genes in *B. quintana*, and to use these to study their contributions to host cell pathology, (2) to develop a cell culture infection model with the mutants to compare innate immune activation in the presence of the bacteria, and (3) to use proteomics methods to analyse the post-translational modification of the putative host cell targets.

METHODS

Our first aim, to develop isogenic mutants, involved introduction of mutagenic plasmids into wild type JK-31, a *Bartonella quintana* clinical isolate. DNA was introduced into the bacteria via conjugation or electroporation. Controls were included for the conjugation (i.e., conjugation into an *E. coli* recipient along with the *B. quintana* recipient). The bacterial recipients were selected on plates supplemented with kanamycin or gentamicin; the *E. coli* conjugative strain was counter-selected with nalidixic acid and cefazolin.

Cell culture models were developed to assess NF-κB activation. NF-κB localisation was visualised with a fluorescently tagged antibody to p65 (RelA), which is transported to the nucleus upon activation.
The localisation of p65 was determined for multiple microscopy fields using an IN Cell Analyser confocal imaging system (GE Life Sciences). The degradation of the NF-κB inhibitor, IKB, was observed by immunoblot of cell lysates at various time points following TNF-a stimulation. To assess cytokine production, cultured cells were stimulated with TNF-a for 1 hour, then cell culture medium was collected and cells were lysed. Both cell medium and lysate were applied to a Proteome Profiler Human Cytokine Array Kit (R&D Systems). Resulting images were scanned, pixel density quantified, and data analysed in Excel. Findings were confirmed by quantitative RT-PCR of selected cytokine genes.

To characterise YopJ- or BepA1-mediated post-translational modifications of host cell target proteins, we cloned the corresponding genes into bacterial expression plasmids and purified the proteins. Both YopJ and its putative target protein, DCUN1D1, were cloned into the pFN2A GST protein expression vector (Promega) and purified using the

MagneGST Protein Purification system (Promega). YopJ-GST was expressed along with multiple chaperone proteins (TakaraBio Chaperone Plasmid set) to improve solubility. As YopJ could not be purified from *E. coli*, we used HEK293 cells transfected with YopJ in the CT-GFP Fusion TOPO mammalian expression vector (Life Technologies).
In vitro acetylation reactions were carried out by combining DCUN1D1-GST, purified from *E. coli*, with lysates of HEK293 cells expressing YopJ. GST-tagged DCUN1D1 was purified and enriched following the reaction, digested with trypsin and analysed by LC-MS mass spectrometry.

RESULTS

Despite concerted efforts, we have been so far unable to generate either a YopJ or BepA1 mutant. Conjugations of the plasmids into *B. quintana* have failed to result in colonies following selection. A range of controls have been included to ensure the plasmids and the conjugative strains are all functional. These experiments were disrupted by the COVID-19 lockdown but have since been restarted. We have developed alternative protocols to try, including minor alterations to standard conjugation procedures and larger changes, such as introducing the plasmids via electroporation. These experiments are still underway.

Although genetic deletions were not obtained, we approached the other aims by transfecting HeLa or HEK293 cells with mammalian expression vectors encoding YopJ or BepA1, or their catalytic mutants. We demonstrated that YopJ, but not the catalytic mutant, inhibits the nuclear localisation of NF-κB following TNF-a stimulation. We are investigating whether this is due to inhibition of degradation of the inhibitor IKB, which binds NF-κB and retains it in the cytoplasm; these studies are still ongoing. We examined the abundance and turnover of other components in the NF-κB activation pathway in the presence of YopJ or its mutant, including the cullins, NEDD8, and levels of IKB phosphorylation, to identify the precise point in signaling that is altered by YopJ.

BepA1 disruption of the synthesis and secretion of multiple cytokines in transfected cells was demonstrated by quantifying cytokine levels in the medium (secreted) or the lysate (synthesised but not secreted). At least 13 cytokines, all regulated by NF-κB, were produced at reduced levels in cells transfected with BepA1, relative to those transfected with the BepA1 mutant. We used qRT-PCR to confirm reduced gene expression of selected cytokines under these conditions.

In developing in vitro acetylation or AMPylation reactions, we have encountered difficulties in purifying soluble, active *B. quintana* proteins from *E. coli.* As an alternative, lysates from BepA1 or YopJ-transfected cells are combined with the tagged host target protein, purified from *E. coli.* Following the reaction, the tagged host proteins are purified and analysed by LC-MS. These experiments are underway and have begun to return promising results.

CONCLUSION

Our data suggest both *B. quintana* effectors suppress immune activation in host cells, though via different mechanisms. The enzymatic activity of the effectors (acetylation for YopJ and AMPylation for BepA1) are critical for their function. Both disrupt cytokine secretion, and we have shown that YopJ does this by inhibiting NF-κB nuclear localisation. We are currently pinpointing the steps in activation and signaling that are disrupted by YopJ. BepA1 also disrupts cytokine signaling, via mechanisms that are still being studied. We have developed a method to carry out in vitro reactions, which will allow further investigation the precise mechanisms of post-translational modification carried out by these effectors.

CENTRE FOR BIODISCOVERY

Victoria University of Wellington

Marine natural products and synthetic analogues as potential new therapeutics [Interim Report]

J MILLER, P NORTHCOTE, J HARVEY, R KEYZERS, O ZUBKOVA

KEY FINDINGS

Marine natural products present with a diverse range of bioactive compounds, some of which may prove useful in treating cancer. Their potency, their selectivity for their targets, and their bioavailability in the body can be modulated by semi-synthetic alterations to the parent compounds. Totally new structures can also be synthesised and modified to the same ends. The successes, however, at finding clinically useful products are extremely limited and the procedures inefficient, requiring many years of research to reach the clinical trial stage. Yet, natural products have remained the most consistently successful source of new drug leads.

OBJECTIVES

The aim of this project was to screen novel compounds and their derivatives isolated from marine animals, algae, or terrestrial plants for bioactivity against human cell lines that serve as model systems for studying disease. New synthetic compounds if available would also be investigated.

METHODS

Cultured cell lines were treated with compounds isolated from diverse organisms or synthesised by chemists to determine if the compounds could prevent cell proliferation or were cytotoxic to cells. A standard cell proliferation assay, the MTT assay, was used to screen the pure compounds for bioactivity, and further attempts to identify the mechanisms of action were made in selected cases.

RESULTS

Most of the screening at Victoria University of Wellington was carried out to determine how effective new compounds were at preventing cancer cell growth in culture. A limited number of new compounds were tested during the course of this grant and the results published. The process is on-going, and we continue to screen new natural products or synthetic drugs as the compounds are made available to us.

In the last year, we have mainly worked on the heparan sulfate (HS) mimetics with Dr Olga Zubkova's laboratory at the Ferrier Institute in Lower Hutt. These compounds inhibit the enzyme heparinase which plays a major role in tumour metastasis and tumour growth factor release. Some new HS mimetics consisting of tetramers constructed around a dendrimer core have been synthesised in Dr Zubkova's laboratory at the Ferrier Research Institute in Lower Hutt and tested in my lab at Victoria University. Dr Zubkova is currently in the process of designing and synthesising HS trimers and dimers of the initial lead compounds to improve their activity. Selected lead HS mimetics are also being tested in other labs in New Zealand and Australia in mouse models of breast cancer with support from MBIE, the Breast Cancer Foundation of NZ, and the Health Research Council. Good results have been seen for myeloma, metastatic breast cancer, and colorectal cancer xenografts. A paper describing some of this research on HS mimetics has been published (Zubkova et al. 2018).

CONCLUSIONS

The search for new compounds from natural sources that are effective against cancer will continue to be pursued despite the very poor success rate. However, the rewards for developing an effective anticancer drug with good potency, bioavailability, and few side effects make the search worthwhile.

Victoria University of Wellington

Identifying and characterizing genetic regulators of ubiquitin-dependent sphingolipid metabolism in childhood Alzheimer's disease and healthy persons

A MUNKACSI

KEY FINDINGS

The proteasomal deubiquitinase enzyme UBP6 is crucial to tolerate the sphingolipid disruption and the protective mechanism against sphingolipid perturbation is possibly occurring via regulation of lipid droplet biogenesis.

OBJECTIVES

Sphingolipids are critical structural and signalling molecules in all eukaryotic cells, that when defective in humans, are involved in the onset and progression of many human diseases. However, the molecular regulation of sphingolipid metabolism is not fully understood. As sphingolipid metabolism is highly conserved from yeast to human cells and the genetic, structural and signalling aspects of sphingolipid are complex, the tractable genetic model yeast has provided most of what is known about sphingolipid metabolism. Notably, post-translational regulation of ubiquitin-dependent sphingolipid metabolism is understudied, albeit this aspect of sphingolipid metabolism has been proposed to underlie common, polygenic diseases such as cardiovascular disease, diabetes, cancer, and neurodegenerative diseases. Here we proposed to identify and characterize the genetic regulation of ubiquitin-dependent sphingolipid metabolism. We used unbiased genomic analyses to identify the deubiquitinase genes that regulate ubiquitin-specific sphingolipid metabolism in yeast and initiated investigations of the molecular processes and associated human diseases impacted by these deubiquitinase genes.

METHODS AND RESULTS

AIM 1: To identify genes that regulate ubiquitin-dependent sphingolipid metabolism. We disrupted sphingolipid metabolism in a library of yeast strains each with a mutation in a different deubiquitinase gene. Sphingolipid disruption was achieved by treatment with myriocin, an inhibitor of the first and rate-limiting step in sphingolipid biosynthesis. Out of 18 non-essential gene deletions and 1 essential gene knockdown, the deletion of UBP6 (ubp6Δ), the yeast ortholog of the human ataxia Usp14 gene, was clearly the most sensitive to myriocin with 95% growth inhibition (Figure 1). In contrast, the other mutant strains were either not significantly different from WT or significantly different albeit with only ~60% growth inhibition.

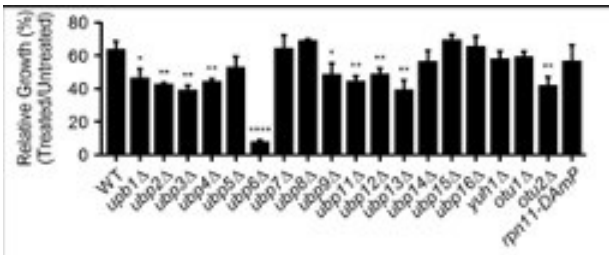


FIGURE 1: The proteasomal deubiquitinase mutant *ubp6Δ* unable to tolerate sphingolipid disruption upon myriocin treatment.

Next, we screened for gene overexpressions that suppress the sphingolipid- specific growth defect of ubp6Δ. We overexpressed a plasmid library of 5,800 essential and non-essential genes in ubp6Δ and evaluated the growth of the strains on media containing galactose (to induce gene overexpression) or glucose (to maintain the normal gene

expression) in presence and absence of a concentration of myriocin (250 ng/mL) that is lethal to ubp6Δ. We identified 48 gene overexpressions that suppressed the sphingolipid-specific lethality in ubp6Δ. We confirmed that these were not generic suppressors of sphingolipid defects as these overexpressions did not suppress myriocin sensitivity in WT. To determine if these 48 genes were over-represented for specific molecular processes, an enrichment analysis was conducted using YeastEnrichr. The mitogen-activated protein kinase (MAPK) signaling pathway was the top hit of the enrichment analysis followed by meiosis and endocytosis. There are four distinct MAPK pathways involved in the response of yeast cells to stress; these are MAPK pathways involved in pheromone response, filamentation, high osmolarity growth (HOG) and cell wall integrity (CWI). The genes that conferred myriocin resistance were all involved in cell wall integrity such as MKK1, MKK2, MPK1 (yeast orthologue of human ERK), PKH1, PKH2 (yeast orthologue of human PDK1), YPK1 and YPK2 (yeast orthologue of human AKT).

Aim 2: To investigate the sphingolipid-dependent molecular processes and human diseases impacted by mutation in the UBP6 deubiquitinase gene. Disrupted sphingolipid metabolism is associated with many human diseases including cancer, neurodegenerative diseases and diabetes. With our identification that UBP6 was a critical gene in responding to disrupted sphingolipid metabolism, we sought to connect UBP6 with molecular processes that are aberrant in human diseases. Lipid droplets are fat-storage organelles composed mainly of triglycerides and cholesterol ester that are maintained at a precise size and number to prevent lipotoxicity (lipid-dependent cell death) associated with cardiovascular disease, neurodegenerative diseases, obesity and diabetes. Here we used fluorescent microscopy to monitor lipid droplets in response to UBP6 deficiency. Using a Nile Red stain specific for lipid droplets, we determined that lipid droplet number was increased in response to UBP6 deficiency compared to WT. This change was only seen with the myriocin treatment, suggesting this disruption in lipid droplet homeostasis is a consequence of disrupted sphingolipid metabolism.

CONCLUSION

Our overall objective was to gain insight into the molecular mechanisms by which eukaryotic cells tolerate changes in sphingolipid metabolism. As defective sphingolipid metabolism is associated with many diseases, additional understanding of ways to buffer these defects provides potential therapeutic targets to treat these diseases. In Aim 1, we determined that specific components of the MAPK signaling pathway, each conserved from yeast to human, are critical means for eukaryotic cells to tolerate defects in ubiquitin-dependent sphingolipid metabolism. Although the exact mechanism on how UBP6 is involved in MAPK signaling pathways is yet to be elucidated, our results predict that UBP6 plays a role in activating the MAPK pathway. In Aim 2, we determined that lipid droplet production is a molecular process that can also be activated in response to disrupted sphingolipid metabolism. Future research will focus on integrating the major results in these two aims, specifically testing if UBP6-mediated MAPK activation is involved in lipid droplet biogenesis in yeast cells as well as human cell models of neurodegenerative and metabolic diseases.

Victoria University of Wellington

Engineering enzymes to protect cellular therapy vectors against activated prodrugs

A SHARROCK

KEY FINDINGS

This research project successfully identified a panel of bacterially derived enzymes capable of protecting bacterial and human cells against DNA damage associated with two artificially synthesised toxin molecules. The identified enzymes will support our ongoing development of cell-mediated targeted chemotherapies, where they could serve to protect gene therapy vectors against chemotherapeutic drugs and prevent premature termination of therapy.

OBJECTIVES

Gene directed enzyme prodrug therapy (GDEPT) is a gene therapy approach involving tumour-specific delivery of a vector encoding a prodrug-activating enzyme. Following systemic administration of a prodrug, the prodrug-activating enzyme converts the prodrug into an active therapeutic molecule capable of destroying surrounding cells within the tumour microenvironment. This can include not only cancerous cells, but also cellular vectors. Destruction of the gene delivery vector can be disadvantageous, firstly because these engineered cells are often costly and laborious to generate, and secondly because it can lead to premature termination of the therapy. The main objective of this project was thus to identify novel enzymes capable of protecting bacterial and human cell-based gene delivery vectors against DNA damage caused by chemotherapeutic drugs.

The initial aim of the project was to survey the literature to identify candidate enzymes and assess their expression in bacterial cells. We next sought to evaluate the level of protection they conferred to *E. coli* cells against two artificially synthesised toxin molecules, which served to model how well they may protect *Salmonella*-based gene delivery vectors. Following validation in bacteria, we also aimed to test the enzymes in an analogous manner in human cell lines to model how well they may protect human cell-based gene delivery vectors.

METHODS

Putative protective enzymes were chemically synthesised and cloned into an *E. coli* expression vector. *E. coli* strains expressing each of the candidate enzymes were generated, and the relative tolerance of each bacterial strain to over-expressing each enzyme were assessed. Bacterial cytotoxicity (IC_{50}) assays were performed to determine the sensitivity of each strain to two toxin molecules, where an increase in IC_{50} compared to an 'empty vector' strain indicated that the candidate enzyme exerted a protective effect against the toxin being tested.

Genes encoding each candidate protective enzyme were additionally cloned into a mammalian cell expression vector to allow for expression and testing in human cells (HEK-293). Stable transgenic cell lines expressing each enzyme were generated and IC_{50} assays were performed in a manner analogous to those performed in *E. coli*, with cell viability monitored by MTS assay. To visually illustrate the protective effect of a repair enzyme, fluorescently tagged versions of both wild-type and protective enzyme-expressing HEK-293 cells were generated. Co-culture of these fluorescent cell lines followed by incubation with a toxin molecule and image capture with a fluorescence microscope allowed us to visualise the relative proportions of surviving cells from each cell line.

RESULTS

Expression tolerance in *E. coli*

Following a literature survey, five candidate protective enzymes (denoted A, B, C, D and E) were identified. Genes encoding each enzyme were cloned into an *E. coli* expression vector and used to transform an *E. coli* expression strain. The *E. coli* expression strain lacks a gene encoding an outer-membrane efflux pump that is responsible for efflux

of many drugs, resulting in increased sensitivity of this strain to the toxin molecules used in this study. Growth assays performed with each strain revealed that expression of the protective enzymes C, D and E was well tolerated, however expression of enzymes A and B substantially reduced the growth rate of *E. coli* cells (Figure 1). Enzymes A and B were therefore excluded from subsequent bacterial experiments.

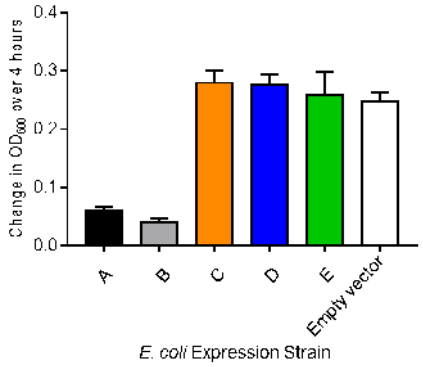


FIGURE 1: Growth rates of *E. coli* strains overexpressing a putative protective enzymes (A-E)

Protection of bacterial cells against two artificially synthesised toxins

IC_{50} growth assays were performed to assess the ability of enzymes C, D and E to intracellularly protect *E. coli* DNA against two artificially synthesised toxin molecules. By comparing the sensitivities of wild-type and protective enzyme-expressing strains to either drug, it was revealed that all three enzymes protected *E. coli* cells against the toxin molecules to some degree, with enzyme D providing the highest degree of protection.

Protection of human cells against two artificially synthesised toxins

Genes encoding each of the five putative protective enzymes were cloned into a mammalian expression vector and expressed stably in HEK-293 cells. The sensitivities of each of these cell lines to the two toxin molecules were assessed by means of MTS cell viability assay. The IC_{50} (concentration of drug required to reduce growth of cells to 50% that of an unchallenged control) for each drug was calculated for each cell line. Each cell line expressing one of the five protective enzymes had reduced sensitivity to both toxins, reflected in their IC_{50} values being between 17.5- to 27-fold higher and 4.4- to 7.2-fold higher than those of wild-type cells for toxin 1 and toxin 2 respectively.

Visually illustrating the protective effect that one of the protective enzymes confers to HEK-293 cells against toxin challenge

Transgenic HEK-293 cell lines were generated that stably expressed either 1) a protective enzyme and GFP, or 2) mCherry alone. These two cell lines were grown in co-culture and challenged with a toxin. Cell survival was subsequently visualised by fluorescence microscopy. Following toxin challenge we observed substantially greater survival of the green, protective enzyme-expressing cells compared to the red wild-type cells (Figure 2). Future work will involve performing similar experiments with mCherry-expressing cells co-expressing each of the four enzyme homologues identified in this study.

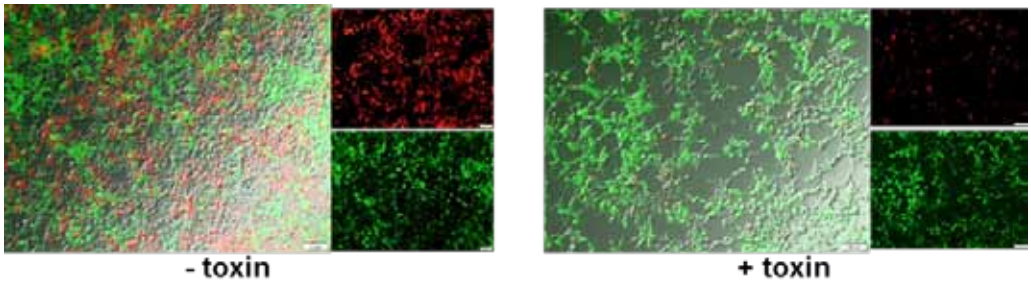


FIGURE 2: Fluorescence microscope images depicting the survival of fluorescent wild-type (red) versus protective enzyme-expressing (green) HEK-293 cells following toxin challenge. Large panels = overlay of brightfield image and mCherry + GFP fluorescence images. Inset panels = single channel mCherry (top) or GFP (bottom) fluorescence images.

CONCLUSION

As a result of this research, we have identified five new enzymes capable of protecting bacterial and/or human cells against two artificially synthesised toxin molecules. Three of these enzymes were found capable of protecting *E. coli*, whilst all five were found capable of protecting human cells. These enzymes show promise for the development of gene therapies in which the DNA of the gene delivery vector is protected from chemotherapeutic drugs. This protection would play an important role in preventing unwanted, early termination of the therapy resulting from self-sterilisation of vector cells.

RESEARCH PROJECT & TRAVEL GRANTS

Research Project Grants 2020

THE FOLLOWING PROJECTS HAVE BEEN APPROVED FOR FUNDING AND WILL BE REPORTED ON IN SUBSEQUENT RESEARCH REVIEWS.

Improving Adherence to a Reduced Carbohydrate Diet for Women with Gestational Diabetes

TUTANGI AMATAITI

Tutangi Amataiti received a grant of $4,107 to undertake research to improve health outcomes of women with Gestational Diabetes (GDM), or diabetes in pregnancy, through a novel dietary intervention to modify carbohydrate intake to reduce excess weight gain in pregnancy. The prevalence of GDM in New Zealand is increasing at a rate of 8-9% per year and is higher among Māori and Pacific ethnic groups. In addition, GDM increases the risk of complications for the mother and baby. These complications are exacerbated by excess weight gain during pregnancy. GDM can also increase the risk of developing Type 2 diabetes for the mother and for the offspring later in life.

Tutangi Amataiti is a Master of Health Science candidate at the University of Otago, Wellington and a Registered Dietitian at Capital & Coast District Health Board.

Δ133p53: A driver of prostate cancer through inflammation

PROFESSOR ANTONY BRAITHWAITE

Professor Braithwaite received a grant of $11,000 to undertake research into prostate cancer. Over 3,000 New Zealand men are diagnosed with prostate cancer annually and although prostate cancer is curable if diagnosed early, there are currently few effective treatment options for patients with advanced disease. Professor Braithwaite's research is aimed at understanding how the immune system is recruited by prostate cancer cells to promote disease progression.

Professor Braithwaite is a Visiting Scientist at the Malaghan Institute for Medical Research and a Professor at the University of Otago. This is a collaboration between the institutes.

Investigating the mRNA expression of genes differentially methylated in the livers of guinea pigs born prematurely, or at full-term

DR DONIA MACARTNEY-COXSON AND DR MAX BERRY

Dr Macartney-Coxson and Dr Max Berry were awarded a grant of $5,165 to undertake research to help understand why individuals who are born as preterm babies have an increased risk of diseases like type-two diabetes in later life. Their research is specifically looking at the liver which is very important for metabolic health.

Dr Macartney-Coxson is a Science Leader at The Institute of Environmental Science and Research, and a member of the Healthier Lives, He Oranga Hauora National Science Challenge. Dr Berry is Director of the Centre for Translational Physiology at the University of Otago, Wellington, and a consultant neonatologist at Capital and Coast District Health Board.

Harnessing innate-like T cells as mucosal adjuvants for novel influenza vaccine development

THERESA PANKHURST

Theresa was granted $9,200 to undertake research to help develop novel influenza vaccine strategies that harness innate-like T cells as mucosal vaccine adjuvants. Theresa's research is currently exploring the adjuvant potential of innate-like T cells to drive potent antigen-specific humoral and cellular immune responses capable of enhancing protection against influenza infection. The aim of Theresa's research is to develop a safe and effective influenza vaccine that can be delivered to the mucosa via the intranasal route.

Theresa is a PhD student in Immunology at Victoria University of Wellington.

Real-time brain health measures in clinically relevant ovine stroke models

FREYA HARRISON

Freya was granted $11,570 for the study of brain health changes within stroke and validation of a new, portable brain health monitoring device. Conventional equipment, such as MRIs, are powerful but inaccessible, and result in only 7% of Kiwi stroke patients receiving active treatment in time. It is believed this device can replace conventional MRI scans, providing stroke diagnostic and monitoring capabilities to rural and impoverished areas to improve their health outcomes.

Freya is a Research Assistant at the School of Chemical and Physical Sciences, Victoria University of Wellington.

The Single-Cell Transcriptome of Rectal Cancers before Chemoradiation Therapy

ANNABELLE GREENWOOD

Annabelle was granted $18,000 to apply a state-of-the-art biomedical technique in rectal cancer research. New Zealand has one of the highest rates of bowel cancer in the world leading to 1,250 deaths per year. Improving treatment strategies in advanced rectal cancer is currently a research priority to improve long term outcomes. The research will involve uncovering the genetic and immune ecosystem of tumours resistant and sensitive to chemoradiation therapy at high resolution.

Annabelle is a PhD candidate in the Department of Surgery and Anaesthesia, University of Otago, Wellington.

Determining population-level genetic regulators of sphingolipid metabolism that contribute to the diversity of cancer progression in individuals

DR ANDREW MUNKACSI

Andrew was granted $8,762 to undertake research to help find novel treatment avenues for cancer and provide insight into a fundamental process that is not completely understood in healthy people. Sphingolipids are critical structural and signaling molecules in all eukaryotic cells that, when defective in humans, are involved in the onset and progression of many human diseases including cardiovascular disease, diabetes, neurodegenerative diseases, and cancer. However, the molecular regulation of sphingolipid metabolism is not fully understood in healthy or diseased cells. Andrew's research is exploring the genes and processes that regulate defects in sphingolipid metabolism that underlie many forms of cancer.

Dr Munkacsi is a translational research scientist at the School of Biological Sciences of Victoria University of Wellington.

The Extracellular Vesicle (EV) Profile of Cardiac Fibroblasts Stimulated with a Pro-inflammatory or Pro-fibrotic Stimulus in vitro

MORGANE BRUNTON-O'SULLIVAN

Heart disease remains the leading cause of morbidity and mortality worldwide and poses a significant health burden to New Zealand. Morgane Brunton-O'Sullivan was granted $15,717 to undertake research that investigates how heart cells influence cardiac repair. More specifically, Morgane is interested in examining how small particles produced by heart cells change in response to stimuli conditions which mimic the microenvironment of the heart following a heart attack. This research will allow us to elucidate how heart cells contribute to disease progression following a heart attack and is a first step in developing biomarkers which aim to measure cell activity.

Morgane is a PhD candidate working in joint collaboration with the University of Otago and Wellington Regional Hospital.

Biomarkers and their Relationship to Acute Brain Injuries in the Emergency Department. The BRAIN Study.

DR ALICE ROGAN

Dr Rogan was granted $12,858 to conduct research investigating the use of blood tests to help diagnose Traumatic Brain Injuries (TBI). TBI can occur from a wide range of traumatic causes such as falls, motor vehicle accidents, sports or violent assaults and is a leading cause of death and disability in New Zealand. People can be diagnosed with mild TBI such as concussion or more severe TBI such as bleeding and swelling of the brain. Most New Zealanders will either have had, or know someone who has been affected by, TBI. At the moment, doctors use their examination skills to decide whether or not someone needs a head CT scan to exclude a more severe injury. This can be really difficult, particularly in people with severe concussion or those who may be intoxicated with drugs or alcohol. Alice's research will explore whether or not blood tests can be used to help decide which patients need a CT scan and which patients may have concussion.

Dr Rogen is an Emergency Medicine Research Fellow within the Department of Surgery at the University of Otago, Wellington and an ACEM Emergency Medicine Registrar at Wellington and Hutt hospitals.

Determining the clinical utility of a monocyte inflammatory score for predicting infarct expansion in patients with Acute Myocardial Infarction (AMI)

DR KATHRYN HALLY

Dr Hally was granted $18,703 to undertake research to identify patients that are at-risk of worse repair after a heart attack. Heart attacks are a leading cause of death and disability in New Zealand and, for those that survive, life after a heart attack is far from free of health complications. The heart starts to repair soon after a heart attack but, in some patients, the quality of this repair process is compromised. Currently, there is no way to identify and therefore treat patients who experience detrimental repair. Kathryn's research investigates the role of monocytes in driving the immune response to a heart attack and the potential for this cell to act as a novel biomarker for predicting worse heart repair.

Dr Hally is a Heart Foundation Postdoctoral Fellow at the University of Otago, Wellington.

Can a positive environment reverse the effects of a maternal viral infection on the brain of the children?

PROFESSOR BART ELLENBROEK AND DR LIFENG PENG

Professor Ellenbroek and Dr Peng were granted $11,000 to investigate how a maternal infection affects the brain of children (in-utero).
The Covid-19 pandemic has shown once again how dangerous a viral infection can be. Perhaps less well-known to the general public is the fact that when pregnant females are infected with a virus, their unborn children have a substantially increased risk of developing mental disorders later in life.

In this project Professor Ellenbroek and Dr Peng, both of Victoria University of Wellington, combine their expertise in neurobiology and biochemistry to investigate all the protein changes seen in the sample exposed to a viral infection. Moreover, they will investigate whether exposure to an enriched environment prevents some (or all) of these changes. Together, their results may pave the way for the development of new therapies for disorders such as schizophrenia, autism spectrum disorders and major depression.

Travel Grant Reports

Note that from March 2020, travel grants were not offered, and in some cases were withdrawn, due to Covid-19 travel restrictions.

ANNA ADCOCK

33RD INTERNATIONAL PAPILLOMAVIRUS CONFERENCE, BARCELONA

Due to the COVID-19 pandemic the conference was postponed until July 2020 and changed to a virtual conference.

Despite the different and unexpected format, it was still a worthwhile event to participate in, and I was able to achieve three research outputs from it: two posters and one oral presentation.

1. He Tapu Te Whare Tangata (the sacred house of humanity): Reaching Under-Screened/Never Screened Indigenous Peoples: HPV Self-Testing

   I co-authored this poster that shared the success of our HPV self-testing RCT in Te Tai Tokerau. The RCT provided evidence that the introduction of HPV self-testing through routine primary care can improve cervical screening uptake for previously under/never-screened Māori women.

2. He Tapu Te Whare Tangata: Exploration of the Cervical Screening Clinical Pathway

   I am the lead author of this poster that showcased our qualitative research associated with the RCT, exploring responses to the HPV self-test among under/never-screened predominantly Māori women. It was one of only three posters that shared qualitative research with Indigenous women (the other two being about research with Aboriginal and Torres Strait Islander women from Dr Tamara Butler and Assoc Prof Lisa Whop and colleagues). This highlights the real need to ensure that Indigenous women's voices are being heard at conferences like IPVC.

3. Indigenous Peoples and HPV: Moving the Agenda Forward

   I helped plan and was a speaker in the conference's only Indigenous workshop (chaired by Prof Bev Lawton and Dr Margaret Heffernan (Australia)). The 90-minute workshop included presentations on: challenges with cervical cancer/HPV and Indigenous peoples; data and monitoring; innovative research; and next steps for implementing the IPVS Indigenous Policy statement. In the innovative research segment, I talked about our body of work He Tapu Te Whare Tangata and the importance of Indigenous led research. Other contributors included: Wendy Dallas-Katoa and Jane MacDonald (Aotearoa); George Wurtak (Canada); and Lisa Whop, Tamara Butler, Megan Smith, and Suzanne Garland (Australia). It was a privilege to collaborate with these people, and to call on the IPVC community to join the International Indigenous HPV Alliance (IIHpvA) in the fight against HPV.

Many thanks again for enabling me to attend and present (virtually) at IPVC2020. It greatly benefited my professional development by allowing me to hone my presentation skills and collaborate with influential researchers and advocates.

DR NAOMI BREWER

2ND WORLD INDIGENOUS CANCER CONFERENCE, CALGARY

A large contingent attended the conference from New Zealand, including Māori and non-Māori kaumatua, leaders, researchers, clinicians and community service providers. Hei Āhuru Mōwai facilitated a meeting of participants ahead of the conference to discuss appropriate Tikanga and to support a whānau approach at the event including manaakitanga, karakia, waiata and the gifting of Taonga.

The programme included a one-day pre-conference workshop which gave me the opportunity to meet with Canadian First Nation, Inuit and Métis Elders to learn about their experiences of becoming knowledge holders, practices and protocols for transferring knowledge, knowledge protection, practitioner and knowledge seeker/client relationships, and traditional health and prevention practices. I think that this experience will help me to build

better relationships with community partners in the future. Presenting the preliminary results of my research enabled me to discuss it with some of the audience members, including a academic who conducts similar research in Australia. As well as meeting people at my presentation I also met several other researchers from similar fields, so those contacts may be beneficial in the future. Presenting my research has helped me to consider which questions arising from it should be addressed next.

The conference included presentations about various cancer screening interventions that were being led by indigenous communities and researchers in close partnership. I think that this type of partnership will increase in New Zealand and it is useful for us to learn about what has, and has not, worked overseas. The studies are becoming more strength-based (not deficit focused) and examining how to empower people in their cancer journeys – in my field this includes enabling self-sampling for cervical-cancer screening and how this can be implemented with appropriate levels of information and support. Reducing the stigma that can be associated with screening is also important, so research is looking at whether humour (for example around faecal sampling for bowel-cancer screening) can be beneficial. There was also reiteration of the importance of on-going monitoring of cancer in indigenous peoples and the associated necessity of accurately measuring who is indigenous.

At the Country Caucus meeting New Zealand was nominated by Australia, Canada and the USA to hold the third World Indigenous Cancer Conference, to be held within the next 2-3 years. Along with this, New Zealand will now be responsible for hosting and maintaining the World Indigenous Cancer Network website (http://www.wicnetwork.org/) and facilitating the International Indigenous Cancer Leadership Group until the next conference. The opportunity to host the next conference in Aotearoa was well supported by New Zealand participants and Hei Āhuru Mōwai will coordinate the initial actions required to secure support from key national organisations.

ALISTAIR BROWN

ENZYME ENGINEERING XXV, CANADA

Enzyme Engineering XXV took place over September 15-19 2019 in Whistler, British Columbia, Canada. Enzyme Engineering is the premier conference in the Enzyme Engineering field and is attended by both academic and industry leaders. The focus of this conference was 'Frontiers in Enzyme Engineering' and sessions within the conference covered a diverse range of topics including synthetic biology, computational protein design and applications of enzyme engineering for pharmaceutical development and industrial biocatalysts.

I was especially interested in the sessions devoted to the development of new tools for enzyme engineering and the plenary lecture by David Baker titled 'The coming age of *de novo* protein design' as he has played a leading role in the development of computational tools to aid protein design, a key theme of my research. Several other preeminent protein engineers such as Joelle Pelletier also spoke, and it was wonderful to be able to meet them in the more informal poster sessions and make connections that may facilitate future collaborations.

The conference also gave me an opportunity to present my work titled "Directed evolution of the non-ribosomal peptide synthetase BpsA to enable recognition by the human Sfp-like PPTase'. This poster focused on the development on the direct screening of a promising new antibiotic target in *Mycobacterium tuberculosis*. PPTases are essential enzymes that are essential for growth and persistence in both prokaryotes and eukaryotes. A potential source of toxicity for this class of antibiotics is the cross-reactivity with the essential human PPTase, which is structurally related to the *M. tuberculosis* enzyme. We have used directed evolution and rational protein design to develop variants of our biosensor that are capable of detecting the inhibition of the human PPTase, an important step forward in developing a complete antibiotic screening platform that will recover *M. tuberculosis*-specific inhibitors. The feedback and discussion I received was incredibly useful for the completion of a manuscript based on this research.

I'm currently employed under a HRC grant that focuses on the development of a new antibiotic treatment for Gram-negative bacteria. A key collaborator on this grant, Dr Janine Copp, is based at the University of British Columbia's Michael Smith Laboratories, located in Vancouver, close to the conference venue. Dr Copp also attended the conference and we were able to find time to have fruitful discussions around the development of this research programme. A manuscript from this research is currently under peer review with the leading Microbiology journal mBio.

JOSHUA LANGE

CD1-MR1 CONFERENCE, OXFORD

On 1 September 2019 I attended the biennial CD1-MR1 conference at Oxford University in the UK. This conference was a specialised meeting that gathered experts in the field of specialised immune cell subsets, mucosal-associated invariant T (MAIT) cells. These cells have been a large focus to immunologists for their potential role in treatments for infectious disease, asthma, allergy as well as targets in the development of new anti-cancer drugs. At this meeting, I was able to present my work detailing how MAIT cells could be activated and in turn enhance anti-tumour responses, a first in MAIT cell research. This provided a great platform that allowed me to interact with experts in the field to gain critical feedback and to strengthen new collaborations with international researchers.

At this conference, the presentations highlighted the extraordinary advances within the field and detailed the use of highly advanced techniques such as RNA sequencing technology that can help us understand the fundamental biology of MAIT cells. This information has been highly valuable as part of my PhD in understanding MAIT cells and how they fit in within the complex network of immune cells in our body. Furthermore, the new emerging data helped supplement and inform my own research allowing me to strengthen, as well as generate new, hypotheses. I was also lucky enough to be given the opportunity to give an oral presentation where I detailed how the use of recently discovered drugs that activate MAIT cells could be used to generate anti-tumour immune responses. When administered as a vaccine, the activation of MAIT cells exerts immune modulating effects within the lungs, where MAIT cells are highly enriched. I showed that this modulation of the lung microenvironment generated favourable inflammatory conditions that when formulated with cancer protein targets resulted in tumour-targeted T cells. This data contributed new knowledge to the field of MAIT cells and how they are potential targets for novel cancer therapies. Moreover, this conference provided amazing opportunities to network with fellow scientists including a social event at the Oxford natural history museum and dinner at an old Victorian college. Overall, attending the CD1-MR1 conference has been an invaluable experience that has been of great benefit to my PhD.

DR JOHANNES MAYER

INTERNATIONAL CONFERENCE OF MUCOSAL IMMUNOLOGY 2019, BRISBANE

The conference brought together the most renowned international scientists from the field of mucosal immunology, with a focus on gut health. This focus echoed my own presentation which highlighted an optimized approach to isolate immune cells from heavily infected intestines.

Especially the keynote lectures by Prof David Artis, Prof Bana Jabri and Prof Richard Locksley fascinated me and being able to relate them to my own findings, in discussions afterwards, was highly rewarding.

I was very happy to see that many PhD students, Postdocs and early career researchers from the US and the UK (including some of my old lab members) came to Brisbane, which allowed us to catch up in person and resulted in many fruitful discussions over lunch and dinner.

I was selected to present my own research with a poster and used that opportunity to get critical feedback from several senior scientists about my work. It was great to see that our findings were judged by many as a significant advance over current techniques and that our results attracted much attention.

In the days leading up to the conference I was also able to visit collaborators at the QIMR Medical Research Institute in Brisbane and plan future experiments and publications.

My attendance of the International Conference of Mucosal Immunology 2019 in Brisbane, Australia was therefore very insightful, highly productive and helped me promote my own research findings to an international audience.

GRETA WEBB

AUSTRALASIAN SOCIETY FOR IMMUNOLOGY ANNUAL MEETING, ADELAIDE

At the annual meeting of the Australasion Society for Immunology (ASI) in December 2019, I presented my work using high dimensional spectral flow cytometry to characterise Dendritic Cells (DCs) in the skin and lymph node. These cells are highly specialised antigen presenting cells, responsible for the initiation of adaptive immune responses. DCs are of interest to immunologists due to their potential to sway the immune response in different directions, making them instrumental to the development of diseases like asthma and allergies.

The work I presented at ASI showed how these cells can be characterised using a high dimensional technique, showing a new way to investigate their behaviour during the priming of T-helper type 2 (Th2) responses, associated with asthma and allergy. This conference was a great opportunity as an early career researcher, as it allowed me to network with the experts in my field and gain valuable feedback and insight on my work.

At the beginning of the conference I was lucky enough to attend a workshop specifically designed with postgraduate students in mind, with presentations on how to get data published, apply for grants, and design experiments. I found this highly valuable for my PhD, as well as my understanding of the world of immunology in general, as these skills are not usually formally taught to researchers.

I attended talks by researchers in the field every day, which showcased the world-leading advances in the field of immunology in Australia and New Zealand. I was also exposed to some of the research being conducted globally by a number of renowned international guest speakers. Being exposed to this emerging science was enlightening, allowing me to see where the field is heading, and how my own work fits into this picture.

Presenting my data in poster format was a valuable experience as well, as I could directly discuss and interact with people showing interest in my work. The use of high dimensional spectral flow cytometry to study DCs is a new approach, showing a lot of potential to increase our understanding of the way these cells behave, and the signals they respond to that lead to inappropriate Th2 priming and allergic disease.

Finally, the ability to network with fellow students, as well as the experts in my field was a privilege, with events fostering this networking every day including a student function, a gala dinner, and even a 5k fun run! Overall, I left ASI 2019 feeling highly motivated to conduct my PhD research, and found it an invaluable opportunity to better myself as a scientist.

JESSICA YANG

AUSTRALIAN SOCIETY FOR PSYCHOSOCIAL OBSTETRICS AND GYNAECOLOGY, 45TH ANNUAL SCIENTIFIC MEETING, MELBOURNE

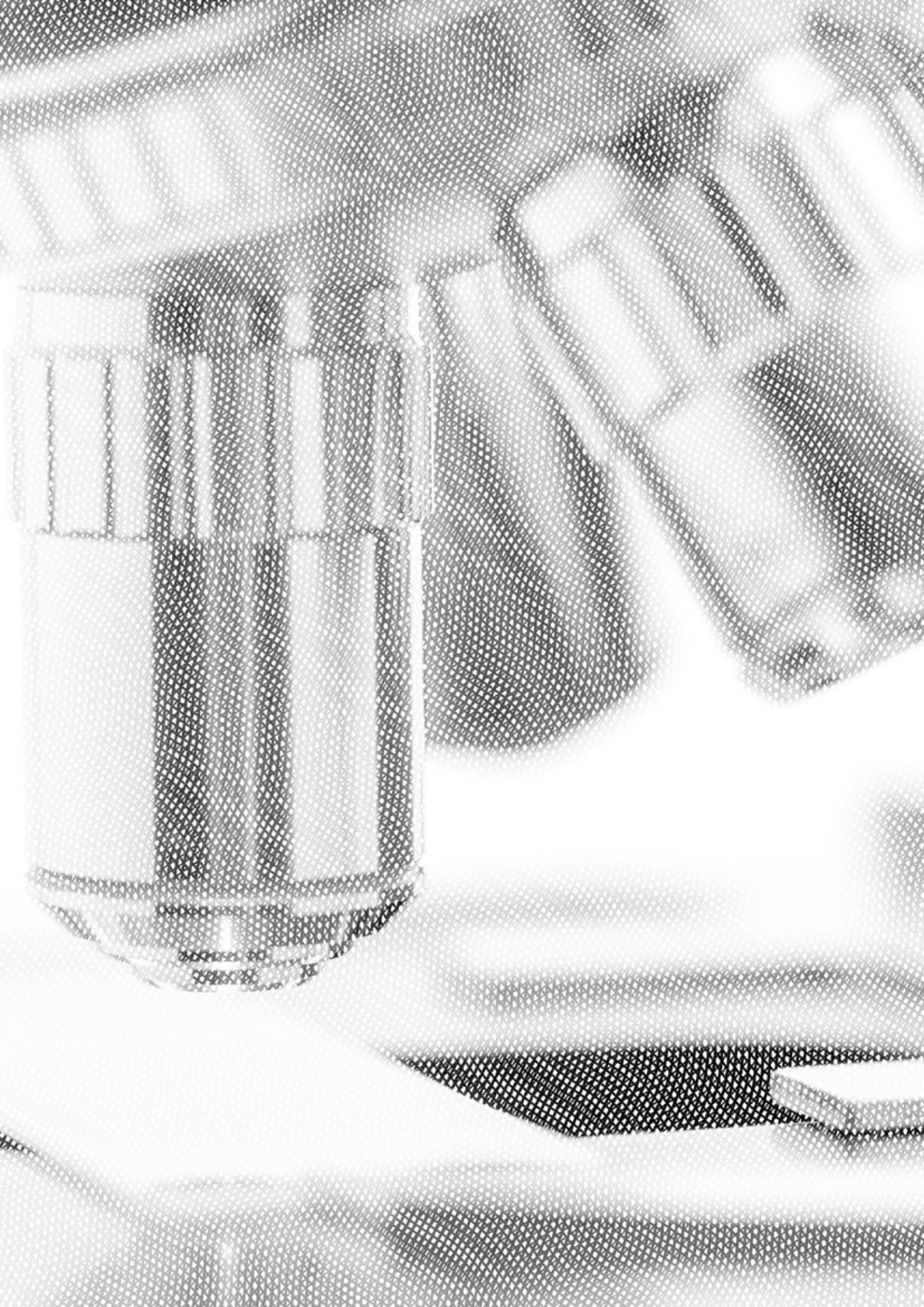
In August 2019, I was privileged to be supported by Research for Life to travel to Melbourne for my first conference presentation at the 45th Annual Scientific Meeting of the Australian Society for Psychosocial Obstetrics and Gynaecology (ASPOG). This was an exciting opportunity to gather with multidisciplinary health professionals and students from across Australasia. A wide variety of important psychosocial issues affecting women's health were discussed, surrounding fertility, sexual health, antenatal and perinatal care, pregnancy loss, and more.

I presented my summer research project, "Provider viewpoints on services and support available for women who have experienced early miscarriage or termination", undertaken with supervision from Dr Sara Filoche and Professor Tony Dowell in the Department of Obstetrics, Gynaecology and Women's Health at the University of Otago Wellington. This was an exploration of the ideas and experience of health providers that work with these women — particularly what these events can mean for women's psychological and social wellbeing — with an aim to identify issues and barriers to inform future health strategies and research. A learning point on my part was how much of a challenge it is to fit fourteen slides and time for questions into fifteen minutes! In this session, I was intrigued to see similar projects conducted by other medical and PhD students but in an Australian context; this to me signifies that we have all independently identified an important and pressing area of research. I enjoyed some engaging discussion about the differences in service availability as well as legal and societal issues in New Zealand compared with Australia; this was a thought-provoking angle for both nationalities represented in the room.

The other sessions I attended were of high interest and educational value to me. In one standout presentation, a fellow medical student spoke on innovations in uterine transplantation which is in its infancy. ASPOG provided me with inspiration to take with me through my career in medicine and, hopefully, in obstetrics and gynaecology, an area for which I have a long-held passion. I gained not only presentation skills and experience, but new ideas and a refreshed curiosity.

My other passion (which marries well with obstetrics and gynaecology) is surgery. I jumped at the chance to also attend the International Surgical Students' Conference (ISSC) held across town on the same weekend, funded by myself with help from the University. This was a great platform for networking with surgeons, meeting many like-minded students, participating in practical workshops such as advanced suturing and perineal repair, representing NZ in a surgical skills competition, and exploring the wonderful city of Melbourne.

I returned to Wellington with skills, stories, and a strong drive to establish a career in research as well as clinical medicine and surgery. I am hugely thankful to Research for Life and the University of Otago for supporting me in this invaluable experience.



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