Division of Health Sciences
Te Wāhanga Matua Mātau Hauora

Project title: The Role of Mitochondrial Dysfunction in Haematological Disease

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Lay summary

Ageing is a major risk factor for many human diseases, however there is limited information on the underlying molecular processes. Mitochondrial dysfunction is one hallmark of ageing, often resulting in less energy production oxidative stress. Previous work had shown higher levels of oxidised mitochondrial protein (peroxiredoxin 3) in the blood of "The Dunedin Study" participants that were aging faster. Furthermore, red blood cells from faster ages were better able to recover after being challenged with hydrogen peroxide. This led to the hypothesis that oxidative stress triggers an adaptive response to produce red blood cells better able to cope with oxidative insult. I used Bristol Erythroid Line-Adult (BEL-A) cells, a cell line that can be successfully differentiated into functional reticulocytes, to explore the effects of mitochondrial dysfunction on the differentiation and the phenotype of resultant cells and to investigate the effects of mitochondrial dysfunction on differentiation. This summer I continued to optimise the conditions for BEL-A expansion and differentiation and the characterisation of cell morphology and surface marker expression as the cells differentiated. I induced oxidative stress by using the mitochondria-targeted redox cycler MitoParaquat which resulted in higher oxidation of peroxiredoxin 3.

Reflective FHR statement

For my summer project I was given the opportunity to continue the research I had conducted during my Bachelor of Biomedical Science (Hons) year in 2023. We predominantly focussed on optimising our protocols and repeating experiments to lay the groundwork for a future publication . I optimised our cytospin protocol as this was a challenging aspectof my Honours project. Optimisation involved testing different staining techniques, methanol fixing times, and testing concentrations of cellular stains. Investigating the methodology was an aspect of my project that aligned with the FHR learning outcomes of engaging in health research methodology as I was able to optimise the protocol with minimal supervision.

Throughout my experience in the FHR programme, I shared resources and facilities with fellow students and researchers which allowed me to appreciate the dynamics within a laboratory and create lasting professional connections. During the summer there were professional development sessions where we interacted with health researchers from all areas and backgrounds of healthcare. This gave us an opportunity to see the impact of health research in a clinical setting and from a public health perspective. This insight was helpful for us to see that our work can make a difference. We also learnt about the importance of integration and consultation of the Treaty within health research. This was very valuable as cultural integration and respect is a particularly important part of our future careers.

I was able to communicate my research at the New Zealand Society for Biochemistry and Molecular Biology Conference at Hanmer Springs in December of 2023. I produced and presented a poster detailing the methods, results, and conclusions from my research project.

Reflecting on my experience in the FHR programme, I have gained a greater understanding of the role of health research in New Zealand and the impact our work can have. I have come to appreciate the importance of laboratory based experiments as a starting point for many health research projects and initiatives. This experience has increased my laboratory skill set, and allowed me to continue to pursue my interest in health research.

Summary of the project

Introduction

There were three main focuses of the my summer project. First was to continue producing differentiating hematopoietic erythroid progenitor cells, and to measure each phase of their differentiation by assessing various surface markers and cell morphologies. Next was to optimise our cytospin protocol in order to increase the amount of cells adhering to the slides, and to establish cell stain concentrations that were effective at distinguishing different components of the cell. Finally, I looked at the effect of induced oxidative stress on peroxiredoxin expression and oxidation by treating differentiating cells with the mitochondrial-targeted redox cycler, Mito Paraquat (MitoPQ).

Methods and Results

To generate a suitable volume of BEL-A cells to differentiate, first a stem cell population must be maintained and expanded in culture. This involved 7 – 10 days of culturing and expanding the stock cells in specialized StemSpan™ media until there was a total of 20×10^6 cells. At this point the cells were transferred to primary media which contains a combination of media additives to encourage proliferation and differentiation. Every two days the cells were counted, then resuspended in the required volume of the respective media. The cells were cultured with various media additives that encouraged differentiation and the loss of stem like properties. After 8 days in culture, the cells were transferred to tertiary media, which increases the holo-transferrin concentration and removes all stem cell promoting additives in an effort to terminally differentiate the BEL-A cells into reticulocytes. At differentiation day 12 the cells were filtered to separate earlier stage progenitors and cell debris, this step would ideally isolate a population of reticulocytes. I characterized the BEL-A cells during erythrocyte differentiation using multiple methods to assess the loss of stem-like characteristics and the acquisition of mature erythroid precursor characteristics. Measurements included surface markers, morphology, cytosolic components, and commitment to the erythroid lineage. At the end of the differentiation culture we aimed to yield a population of isolated reticulocytes which we could challenge with hydrogen peroxide and assess their peroxiredoxin 2 reducing efficiency. This would give an indication of how capable the cell was at mitigating the effect of hydrogen peroxide, and likely other intracellular oxidants. Characterization of basal measurements of BEL-A differentiation are important to establish as they can be used as a reference when measuring differentiation markers in BEL-A cells following induced oxidative stress.

The second project focus was to optimise our cytospin technique to most clearly showcell morphology throughout differentiation. As hematopoietic stem cells differentiate through the cellular subtypes, the orientation of the nucleus is a key morphological indicator of the differentiation phases. We expected the nucleus to centrally condense before localising to one side of the cell, then be expelled during the final phase of maturation into a reticulocyte. Using haematoxylin, a DNA stain, we were able to identify the nucleus within the cells at each stage of differentiation to confirm the successful progression of the cells to reticulocytes. We identified three experimental variables that were likely to have the biggest impact on image quality. Dye concentration, fixative product and timing, and the use of poly-L lysine. We concluded that the best cytospins were a result of diluting the cytoplasm stain 1:2, fixing the cells in methanol for 10 minutes, and coating the slides in poly-L lysine for 30 minutes prior to centrifuging the cells onto the slide.

During my honours project we investigated the effect on peroxiredoxin 3 oxidation after MitoPQ treatment in BEL-A cells, but were unable to repeat this experiment due to time constraints. MitoPQ is a mitochondrially targeted redox cycler that targets Complex 1 of the electron transport chain and increases the amount of superoxide within the mitochondria. We used immunoblotting techniques to assess the potential adaptive response of the cells to oxidant exposure. Preliminary results suggested that the cells upregulate the expression of peroxiredoxin 3 and that a significant proportion were oxidised. This suggests that the cells were compensating for increased oxidative stress. We treated the cells with 1 µM of MitoPQ every two days, with treatment beginning two days before differentiation commenced. This work will enable us to get a more comprehensive overview of the effect of mitochondrial oxidative stress on erythroid differentiation and adaptive response.

Conclusions

The work I completed during the FHR has produced more evidence to support the successful characterisation and differentiation of BEL-A cells in our laboratory and provided preliminary data for future work. These findings will support the ongoing research into ageing by the Mātai Hāora - Centre for Redox Biology and Medicine.