First Research Period Report: Understanding Heart Muscle Organisation in Dilated Cardiomyopathy
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Natalja Nassonova, University of Leeds

Introduction

Cardiomyopathies are diseases of the muscle tissue of the heart (myocardium), which result in structural and functional abnormalities. Dilated cardiomyopathy (DCM) accounts for 60% of all cardiomyopathies, representing the most common form of heart muscle disease (Mitrut et al., 2018). The molecular basis of most DCM cases is unknown (Roura et al., 2017). However, approximately 40% of patients have familial DCM with an identifiable genetic cause (Giri et al., 2021). In this condition, the left ventricle of the heart becomes enlarged (dilated), the muscle wall becomes thinner, which gives the heart a more rounded shape and affects its ability to pump the blood around the body (Burke, 2015). In comparison to other histochemical processes, immunohistochemical methods enable better identification and quantification of the myocardial cells (Kühl et al., 1995). The chronic inflammatory process is manifested histologically as a sparse, diffuse lymphocytic infiltration of the myocardium. Most commonly, dilated cardiomyopathy is caused by auto-immune diseases (e.g. rheumatoid arthritis, HIV), metabolic diseases (e.g. Diabetes mellitus), viral infections (e.g. viral myocarditis), toxins (alcohol or cocaine), chemotherapy, as well as mutations in the genes encoding titin, lamin A, phospholamban, RNA Binding Motif *Protein*-20, Sodium Voltage-Gated Channel Alpha Subunit 5 (Rosenbaum et al., 2020). Currently, dilated cardiomyopathy can be treated with different medications (e.g. diuretics, ACE inhibitors, beta-blockers, Angiotensin 2 Receptor Blockers) and managed using such devices as pacemakers and ICDs. Additionally, lifestyle management can reduce the effects of DCM. However, if the this is not sufficient to control symptoms, a heart transplant might be required (Cardiomyopathy UK, 2021). Therefore, a better understanding of structural alterations caused by DCM might lead to the development of novel treatment methods.

Research Experience

I have conducted my research project on investigating heart muscle organisation in normal heart muscle tissue and heart tissue from patients with dilated cardiomyopathy. The first three weeks of my project involved expressing, extracting, purifying and labelling Affimers for staining. Affimers are proteins that bind to target proteins with affinity in the nanomolar range. These affinity reagents have increased stability and reduced size compared to antibodies, are tolerant to a range of temperatures and pH, are efficiently expressed in *E.Coli* and mammalian cells (Tiede et al., 2017). The last three weeks were dedicated to taking, interpreting, and comparing microscopic images of healthy and diseased myocardium samples stained with Affimers and antibodies. Myocardium samples from four patients and four donors were used.

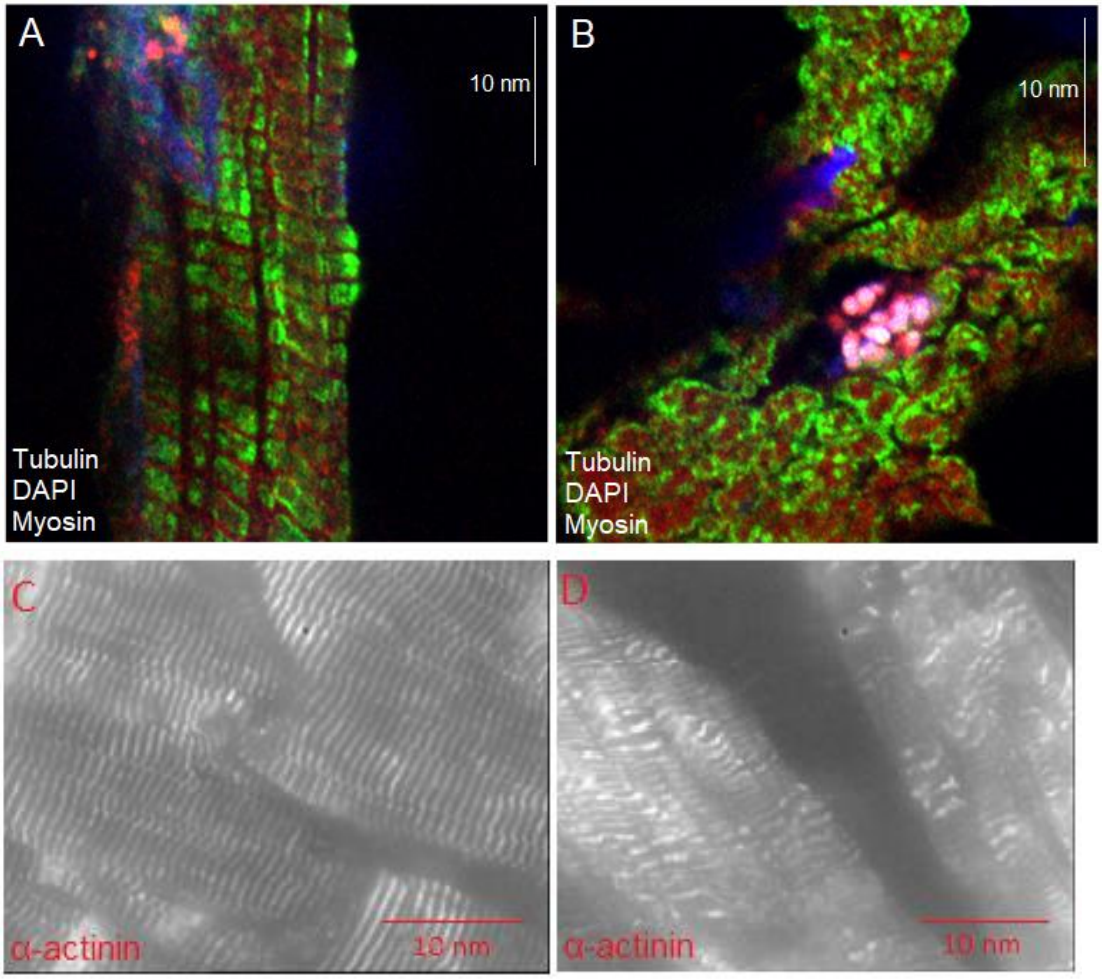

Figure 1: Staining of tubulin with an Affimer to tubulin (red), nuclei (blue) and myosin (green) A. Shows a longitudinal section of normal heart muscle and B shows a cross section of heart muscle tissue affected by dilated cardiomyopathy. Tissues are sex and age matched. C and D: Low power images of normal [C] and dilated cardiomyopathy [D] tissue stained for α-actinin, which shows the stripy appearance of muscle sarcomeres.

As a result of my research, I have identified a repeating striped pattern along the length of the muscle cell (sarcomeres) in normal muscle (Figure 1A)". In dilated cardiomyopathy (shown here in cross-section), most of the myosin staining (in green) is at the edges of the cell and sarcomere appears disorganized with an aggregated Z disc (Figure 1B).

The same is evident from low power images of tissue stained for α-actinin. In longitudinal sections, sarcomeres in myofibrils are arranged in series (end to end) which gives a striated pattern to cells from one end to the other (Figure 1C). The tissue affected by Dilated Cardiomyopathy lacks clear structural pattern (Figure 1D).

I have compared the results obtained with different Affimers and antibodies to identify the most effective combinations, as well as alternative staining options that I can utilise in my second research period. I have started to find differences between normal and dilated cardiomyopathy tissue that I can characterize.

Conducting my research project has been an exceptional opportunity that has helped me to upscale my ability to conduct and interpret laboratory experiments, challenge and develop my scientific thinking and keep myself accountable for conducting the significant part of the project independently. I can say with confidence that, as a result of the 6 weeks I spent in Michelle Peckham's laboratory, I advanced my scientific skills more than I have during all online laboratory practicals we have had throughout the last academic year. My rapid progress is not only explained by the skills I acquired while working with Affimers and staining muscle tissue. A large part of it is the result of communication with other laboratory members, who demonstrated their ongoing projects and shared their experiences of working in academia, as well as the confidence I gained as an independent member of the laboratory. In the beginning, I was very cautious not to add a wrong substance or mix reagents in wrong proportions. I look forward to the second research period to capitalise on the skills and experience I have obtained to delve deeper into understanding the differences in myocardial structural patterns in dilated cardiomyopathy.

Personal and Professional Development

Having conducted the research project over the summer, I feel prepared and confident to go to laboratory classes in person next year and deliver exceptional academic results. After the summer of investigating heart muscle organisation in normal and dilated cardiomyopathy samples, I have not only honed my laboratory skills, including making lysogeny broth agar to grow *E. coli*. and running SDS-PAGE (sodium dodecyl sulphate–polyacrylamide gel electrophoresis) to analyse proteins in addition to my main experiments, but I also learned to organise myself independently as a researcher. The latter is perhaps the most important and difficult skill one can ever develop. I learned to formulate and address questions and doubts I have about an experiment or a concept, perform experiments independently, navigate the lab to find reagents and equipment required for my experiment, follow my schedule and communicate effectively with other lab members and identify when I need help. I'm sure these are the skills that are going to be useful throughout my degree because the project incentivized me to become a more perseverant and self-reliant individual. In addition, I appreciated the opportunity to see the University of Leeds from a different perspective. Before my first research period, I knew staff members, lecturers, course mates, and fellow international students. But I had hardly met PhDs and postdocs working in the same area as myself and saw what their workdays look like. Hardly could I imagine I would ever be taught by those people in a real active laboratory environment. This experience has significantly accelerated my understanding of scientific work, my degree subject, and my potential career. I am grateful to have the knowledge, connections, and experience at an early stage of my academic journey.

Leadership Development

I identified 3 leadership skills that I have gained by the end of my first Laidlaw research period. I will extrapolate what the skills are and how I developed them in the next few paragraphs.

Able to navigate new and foreign situations. Midway through my project, I realised that I had run out of an Affimer to Z-band alternatively spliced protein (Zasp6), which was one of the Affimers I was testing in my research. Due to time constraints, it was not sensible to express the Affimer once more. Therefore, the postdoc I was working on the project with and myself arranged a meeting to come up with an alternative solution. After completing some online research and discussing different options, we concluded that it would be best to substitute the Zasp6 Affimer with A41025, an antibody to myosin, and an antibody to α-actinin for staining. Although in the end we obtained clear images using the antibodies, initially when I had to recognise that a part of the project is not going to go according to our plan A, I felt stuck. Before this occasion, I used to think the project plan and protocols were rigid and amending them was equal to changing the project itself. This summer, I learned that it is important to maintain a high degree of flexibility and react quickly and efficiently to changes. After all, it is an opportunity to make your project even better.

Knowing own limitations and acting accordingly. At the end of my first research period, I concluded that, as a member of any team, you have to be dependable, realistic, and identify when you need help. Although I had a high degree of autonomy throughout my project, I still had to deliver results on time to ensure the overall progress of the project on dilated cardiomyopathy. Therefore, when asked by when I will be able to finish X or analyse Y, I had to learn to give realistic estimates, bearing in mind my capabilities, personal circumstances, and other experiments I had to complete.

Learning continually. That was a “further advanced” skill of mine. I aspire to be a life-long learner and am interested in developing my knowledge in different fields. Michelle’s laboratory is based in the Astbury Centre, where interdisciplinary research takes place at the University of Leeds. I expanded my knowledge on interdisciplinary research not only in the laboratory I was working in but also by reading posters located in the Astbury building and communicating with scientists from other laboratories.

Future career and educational plans

When it comes to planning my future, I prefer to thoroughly plan only the next 1-2 steps and execute them to the highest standard. After that, it is possible to assess whether I am moving in the right direction for myself.

As a professional, I would like to work directly with people and/or for people. Frequent communication and teamwork are inspiring and fulfilling for me. My future career plan is to join a successful company that has plenty of developmental opportunities for its employees, such as Unilever or GSK. I'm interested in seeing how large businesses are organised and gaining work experience in an R&D department. I also want to gain a comprehensive understanding of an academic career during my journey as a Laidlaw scholar. I assume that based on these two work experiences I will be able to choose between staying in academia or pursuing a career in industry after graduation.

My alternative plan is to pursue a Ph.D. after my BSc degree. This decision will depend on two things: my complete reflection on the research experience after the Leadership In Action period next summer and whether the available Ph.D. projects will be within my scope of interest. I'm interested in human microbiome, Diabetes and dilated cardiomyopathy research and would like to participate in expanding scientific knowledge in any of these areas.

Most importantly, I would like to create a rewarding and impactful career for myself to ensure that there is at least one person in the world each day who can benefit from my work.

Supervisor

Natalja has been a hard-working, reliable student in the laboratory. As she started, she had a major challenge to get up to speed with laboratory work, given that all the practicals she has had in the first year have been online. It's very different to be in a real laboratory setting, where the first thing you have to learn is how to use a basic tool, the pipette! Not only that but of course we have had the challenge of undertaking laboratory work while keeping to strict safety guidelines as a result of COVID (strict mask wearing, 2 metre social distancing etc). Given these challenges, Natalja has done really well. She has learnt how to conduct experiments independently, and she has learnt a wide range of techniques from protein expression and purification, to labelling tissue sections and microscopy. The quality of her work has been very good, and I was also able to help her by taking some confocal images of her sections, which were stained well. I think Natalja has summed up her experience, and the challenges of working in the lab, really well in the sections above. Moving forward, I think she has already identified the key area that she can develop further, which is to be flexible in her thinking, so that she can cope with failures by thinking of alternative strategies to make her research a success, and we look forward to welcoming her back for her second research period.

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