University of Glasgow | College of Medical, Veterinary & Life Sciences

Head of College Scholars List Scheme

Summer Studentship

Report Form

This report should be completed by the student with his/her project supervisor. It should summarise the work undertaken during the project and, once completed, should be sent by email to: jill.morrison@glasgow.ac.uk within four weeks of the end of the studentship.

1. Student

   Surname: Catriona Forename: James

   E-mail address: 2016973J@student.gla.ac.uk

2. Supervisor:

   Surname: Stephen Forename: Yarwood

   E-mail address: Stephen.Yarwood@glasgow.ac.uk

3. Research Project Report

   3.1 Project Title (maximum 20 words):

   Hormonal Control of Mesenchymal Stem Cell Differentiation into Fat and Bone

   3.2 Project Lay Summary (copied from application):

   An increased risk of osteoporosis and bone fracture is an underappreciated complication of Type 1 diabetes mellitus (T1DM) and certain drug treatments for Type 2 diabetes mellitus (T2DM). The underlying pathological link is not yet known but it may lie in the balance of differentiation of bone marrow mesenchymal stem cells (MSCs), favouring fat development (adipogenesis) rather than bone (osteogenesis). With the aim of understanding these interrelationships, our aim is to determine the molecular control of osteogenesis and adipogenesis during the diabetic state and/or diabetic drug treatments. These studies will pave the way to the development of bone-regenerative therapies to treat disorders of bone formation associated with diabetes.

3.3 Start Date: 2/7/14 Finish Date: 28/8/14

3.4 Original project aims and objectives (100 words max):

The original aim of the project was to understand the molecular and cellular basis of the control of mesenchymal stem cell (MSC) differentiation into bone (osteogenesis) or fat (adipogenesis) in the context of diabetes and anti-diabetic drug treatment. The current hypothesis is that these processes are regulated by AMPK (AMP-activated kinase)-signalling via the control of Runx2 and PPARγ transcription factors. Therefore the objective of the project was to determine the roles of these pathways in the control of MSC differentiation in response to osteogenic and adipogenic stimuli, by using a murine model of pluripotent stem cell differentiation.

3.5 Methodology: Summarise and include reference to training received in research methods etc. (250 words max):

Whether adipogenesis or osteogenesis will take place is determined by the activation of transcription factors. The transcription factors used to indicate fat or bone growth were Runx2 (an osteogenic transcription factor), PPARγ (adipogenic) and C/EBP, which regulates both osteogenic and adipogenic transcription factors. Their activity varies depending on whether osteogenic or adipogenic media is used. Different anti-diabetic treatments were used to simulate adipogenic or osteogenic environments: pioglitazone and IID (insulin, IBMX, and dexamethasone) are osteogenic, and AGD (ascorbic acid, glycerophosphate, and dexamethasone) is adipogenic. To explore the role of AMPK in controlling differentiation, CH310T1/2 cells were stimulated with each drug treatment in the presence or absence of AMPK activators (metformin and A76) over two nights. The activity of the transcription factors were measured by a luciferase assay. By tagging them with the luciferase enzyme and measuring the level of luminescence in a luminometer, it is possible to measure the level of activation of the transcription factors. Western blotting was also used to determine the effects of PACC, TACC, Runx2 and PPARγ, in order to investigate the mode of action of metformin. CH310T1/2 cells were stimulated with the same treatments with metformin and A76 as for the luciferase assays. The effects of PACC, TACC, Runx2 and PPARγ can then be seen by harvesting the protein and Western blotting.

3.6 Results: Summarise key findings (300 words max). Please include any relevant tables or images as an appendix to this report:

The luciferase activities seen for the different transcription factors in the different treatments with the AMPK activators were compared relative to the control, the CH310T1/2 cells with no treatment. Runx2, the osteogenic transcription factor, showed increased activity in the IID and AGD treatments, but little change in the pioglitazone. With the addition of both of the AMPK activators, there was a drastic increase in Runx2

activation, and this increase was seen across all three of the treatments. The adipogenic transcription factor PPARγ also showed different activity in some of the treatments. Pioglitazone and IID both increased PPARγ activity; however AGD had no real effect. The presence of the AMPK activators increased PPARγ activity in all cases, although not to the same degree as for Runx2. The transcription factor C/EBP is an upstream regulator of both Runx2 and PPARγ, so can encourage either osteogenesis or adipogenesis. C/EBP activity was seen to increase across all of the media. This increase was further enhanced by the presence of the AMPK activators, although again not to the same extent as for Runx2.

The Western blots yielded inconclusive results. Although they showed a slight increase with the IID treatment, the blots would need to be repeated a few more times in order to obtain more data and achieve conclusive results.

3.7 Discussion (500 words max):

The effects of different osteogenic and adipogenic media on MSCs and osteogenesis and adipogenesis were found to be complex. In the absence of AMPK activators, Runx2 is an osteogenic transcription factor that induces osteogenesis in both osteogenic and adipogenic media. The presence of metformin and A76 greatly increases Runx2 activity in each treatment, and therefore enhances osteogenesis, again independently of an osteo- or adipogenic environment. The adipogenic transcription factor PPARγ was active and encouraged adipogenesis in the adipogenic media, pioglitazone and IID. However PPARγ showed no significant change in activity in AGD, so adipogenesis seems to be inhibited within an osteogenic environment. In the adipogenic media, the AMPK activators enhanced adipogenesis relative to no treatment, although adipogenesis took place to a lesser extent than PPARγ alone in pioglitazone and IID. Although C/EBP activity increased in all the treatments and with the AMPK activators, it is a regulator for both Runx2 and PPARγ, therefore it is difficult to determine whether or not it had an effect on osteogenesis or adipogenesis. However, since both Runx2 and C/EBP showed increased activity in AGD, whereas PPARγ appeared to show no effect, it is possible that in an osteogenic environment C/EBP enhances Runx2 and inhibits PPARγ, and so not only is osteogenesis encouraged, but adipogenesis is inhibited. The action of the AMPK activators, metformin and A76, is regulated by the treatments and media used, and whether or not it is an adipogenic or osteogenic environment. Overall AMPK activators seem to enhance osteogenesis and supress adipogenesis, even in adipogenic media, so therefore seem to play a big part in controlling MSC differentiation towards bone formation.

4. Reflection by the student on the experience and value of the studentship (300 words max):

The project was a great learning opportunity. From carrying out the experiments, the guidance from Dr Yarwood and his colleagues in the lab, and from background reading, I was able to learn a great deal about AMPK signalling pathways and transcription factor activation

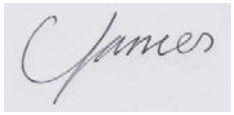
specific to MSC differentiation and to the project. In addition, I was able to build upon previous knowledge and learn of its practical applications. I was able to vastly improve my current lab skills and become comfortable with basic lab protocols, as well as learning new skills and techniques. By the end of the project I felt comfortable carrying out these new techniques, and that I can now carry out my existing skills to a much higher standard. The project has given me more confidence in the lab, as well as increasing my knowledge and understanding of cell signalling. It has been a fantastic opportunity to learn in depth about a topic which I otherwise might not have studied.

Overall I think the studentship is an extremely valuable opportunity. It is a great way to gain some general lab experience. It has allowed me to gain an insight into what the day to day running of a lab consists of, and into what a career in research can be like. It has shown me what sort of problems you can encounter, and how you need to adapt and change your experimental plan or hypotheses depending on the results you obtain. I thoroughly enjoyed my time on the project, and it has definitely encouraged me to pursue a career in scientific research.

5. Dissemination: (note any presentations/publications submitted/planned from the work):

   A publication is planned from the work carried out here.

6. Signatures: Supervisor  Date 23/9/14

   Student  Date 19/09/14

APPENDIX – Luciferase Assay Results