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

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2. Supervisor:

   Surname: Shiels Forename: Paul

   E-mail address: paul.shiels@glasgow.ac.uk

3. Research Project Report

3.1 Project Title (maximum 20 words):

The association of exosomal markers of ageing with socioeconomic status and chronic inflammation.

3.2 Project Lay Summary (copied from application):

Exosomes are small membranous extracellular secretory vesicles containing proteins, coding and non-coding RNAs, as well as multiple signalling molecules that can be used as a source of potential circulatory biomarkers. Whether exosomes can modulate ageing processes and hence organismal fitness, and how this may be affected by psychosocial and lifestyle factors remains to be determined. This project has sought to identify exosomal signatures of ageing correlating with socio-economic, dietary, lifestyle and psycho-social determinants of health span and to relate these to the presence of morbidities in a general population cohort.

3.3 Start Date: 01/06/2016 Finish Date: 29/07/2016

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

The primary aim of this project is to determine the suitability of exosomal non-coding RNAs as biomarkers of ageing by investigating expression levels of exosomal microRNAs modulating key pathways of cellular and tissue ageing in individuals from extremes of socio-economic backgrounds of biological ages in the MRC 20-07 general population cohort.

Due to the newfound link between dietary phosphate intake and premature ageing in a cohort from an overlapping demographic, a secondary aim of this project was to examine the relationship between serum phosphate levels and biomarkers of ageing. However, due to time constraints I was unable to complete this investigation.

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

Before handling clinical samples, initial training was undertaken in basic techniques:

1. Culturing primary mammalian cells

   I had to examine cell growth, detach adherent cells using trypsin, passage cells and harvest nucleic acids and protein from them. I was also taught how to clean and maintain the sterility of the equipment used.

2. Exosome isolation

   Exosomes were extracted from bovine serum using the Exosome Precipitation Kit. The exosomal pellets precipitated from the clinical samples were kept in 0.5 mL TRIzol and homogenised before RNA was isolated from them.

3. DNA extraction

   DNA from the cultured cells was extracted in two different ways – with the Maxwell instrument using the Maxwell DNA Purification Kit and with the TRIzol reagent according to the manufacturer’s instructions. Homogenisation of tissue and extracting DNA from it was also practiced.

4. RNA extraction

   RNA was isolated from cells in 1 mL TRIzol according to the standard protocol and from exosomes in 0.5 mL TRIzol following a revised protocol that uses GlycoBlue to help the precipitation of the small quantity of RNA.

5. Protein extraction

   Protein was extracted from the cultured cells with the TRIzol reagent according to the protocol provided.

6. Nucleic acid purification

   DNA and RNA were purified and concentrated using ZymoSpin columns following the instructions provided. The quality and quantity of the nucleic acids was measured by NanoDrop.

7. RT-PCR and qPCR

   RT-PCR was performed either using random or specific primers and expression of different genes was analysed with qPCR.

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

MicroRNA analysis:

1. Exosome isolation

   56 serum samples from the MRC 20-07 cohort had already been collected before the start of my project. Exosomes were precipitated successfully using the Exosome Precipitation Kit (pellets were visible). Samples were stored in 0.5 mL TRIzol and the pellets were homogenised.

2. RNA extraction

   RNA was successfully isolated from the exosomes in 0.5 mL TRIzol according to the revised instructions including the use of GlycoBlue. The organic phase (containing DNA and protein) was retained for further analysis. Quantity and quality of the RNA was

measured using NanoDrop (Appendix, Table 1) and dilutions of 2.5 ng/ µl in 50µl were prepared for use in subsequent analysis.

3. RT-PCR

   For each sample, cDNA synthesis was successfully carried out for three targets – miR-34a, miR-125b and miR-155, and for the endogenous control, U6 snRNA. Small RNA Assay Kits which contain specific primers that recognise the unique hairpin structures of different microRNAs were used.

4. qPCR

   Small RNA Assay kits were used to run qPCR for targets miR-125b and miR-155 with endogenous control U6 snRNA (Appendix, Figure 1). As this experiment is a part of a bigger study looking into more microRNAs, analysis of the results will be carried out in the future using the $\Delta\Delta C_T$ method.

Serum phosphate analysis:

1. 2220 serum samples from the MRC 20-07 cohort had been collected before the start of my project and I divided them into aliquots of 3x100µl and 2x500µl for further analysis. Phosphate levels will be measured using the Randox Inorganic Phosphorous (PHOS) kits.

3.7 Discussion (500 words max):

Exosomes are extracellular secretory vesicles (30-100nm) which originate from multivesicular bodies within the cell. Their contents vary across cell types and are also dependent on the factors that elicit their secretion (e.g. stress conditions). The focus of this project on them has been warranted by the increasing interest in their role in cell signalling and their ability to modulate cell behaviour (De Toro et al, 2015). Exosomes can accomplish that through their messenger and microRNA contents, which allow them to affect the expression of genes that take part in pathways regulating metabolism, inflammation, autophagy and cell cycle progression in the cells that 'receive' them (Shiels et al, 2016).

In the context of ageing, this offers an explanation of how ageing of one organ can affect distant tissues. Moreover, the ability of different stressors (e.g. nutrient deficiency, hypoxia) to change exosomal contents in a manner that impacts the 'receiving' cells negatively indicates a mechanism for stress-induced acceleration of ageing (Shiels et al, 2016).

Therefore, one of the goals of this project was to identify the miRNA signatures of exosomes from serums of patients from different socioeconomic backgrounds and evaluate their suitability to act as biomarkers. The miRNAs that were investigated were selected due to their association with important cell pathways that can be dysregulated in ageing.

In terms of cellular senescence, miR-125b was found to regulate cell cycle progression by modulating the expression of the CDKN2 locus (McGuinness et al, 2016) as well as p53, which is important not only in cancer, but also in ageing due to its involvement in stress response and apoptosis (Le et al, 2009). MiR-125b can also upregulate various pro-inflammatory genes (e.g. Bcl2 family members), linking low-grade chronic inflammation with ageing (McGuinness et al, 2016).

MiR-155, on the other hand, has been found to be downregulated with ageing (Jung and Suh, 2014). Moreover, it was discovered that one of its targets is a subunit of PI3K – a kinase involved in the IGF-1/GH signalling pathway which is the nutrient sensing mechanism of the cell and therefore directly related to regulation of cell metabolism and autophagy (Hooten et al, 2010).

Since recently a link between phosphate intake and accelerated ageing was established (McClelland et al, 2016), this project was also set out to investigate how phosphate levels might correlate to circulating biomarkers of ageing. Hyperphosphatemia is associated with accelerated ageing due to phosphate's ability to form calciprotein particles, which are toxic to cells. They can also cause vascular calcification, which has been linked with secretion of exosomes with altered contents by vascular smooth muscle cells (Kapustin and Shanahan,

2016). Therefore, the levels of certain miRs in these vesicles may be used as markers of ageing.

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

   I am extremely grateful to have been granted this studentship, as the experience I have gained working in a professional lab has been invaluable. The opportunity to observe, learn and practice laboratory techniques like nucleic acid preparation and PCR set-ups, as well as culturing mammalian cells which was not even a part of my project is something that I could not have done anywhere else. Being able to observe how experiments are designed and how data needs to be analysed has also shown me the importance of bioinformatics.

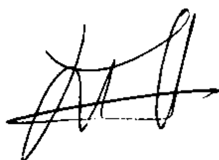
   Working in Prof. Shiels' group was a great experience not only because of the practical work I engaged in but also because of how inviting and willing to help and teach me everyone was. I am especially grateful to Dr. McGuinness, who taught me many of the techniques I had to use as well as how to resolve any problems that arose while I was performing them. Moreover, being able to talk to the technicians, Master's and Ph.D. students about the nature of their work and the types of issues they have to deal with has given me insight into what it is actually like to have a career in academia. Despite the setbacks I experienced and the lack of time to finish the second goal that we set out to accomplish with this project, this has been an invaluable learning experience as it revealed to me how scientific work is carried out in a professional setting.

   Finally, I would like to express my gratitude to Prof. Shiels for taking me on and giving me the opportunity to work in his research group and the Head of College Scholar's Scheme for allowing me to take it.

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

   I will be a co-author on at least two forthcoming publications resulting from my time in the Shiels lab.

6. Signatures: Supervisor Date 24/8/16

Student  Date 18/08/2016

Appendix

Sample	Concentration (ng/µl)	$A_{260/280}$	$A_{260/230}$
1	10.8	1.58	0.59
2	10.1	1.32	0.36
3	8.9	1.56	0.68
4	11.0	1.62	0.40
5	14.0	1.61	0.37
6	16.7	1.51	0.71
7	17.1	1.67	0.62
8	29.6	1.69	0.57
9	13.7	1.40	0.13
10	16.2	1.45	0.61
11	25.3	1.48	0.25
12	40.5	1.59	0.47
13	20.7	1.73	0.67
14	31.5	1.59	0.48
15	44.3	1.52	0.39
16	38.3	1.60	0.62
17	24.5	1.62	0.66
18	13.5	1.38	0.73
19	28.4	1.52	0.49
20	42.3	1.54	0.24
21	17.1	1.53	0.28
22	53.4	1.63	0.21
23	50.5	1.64	0.08
24	31.6	1.62	0.53
25	34.2	1.67	0.68
26	22.2	1.55	0.15
27	42.9	1.71	0.58
28	20.0	1.57	0.71
29	30.2	1.61	0.61
30	23.4	1.59	0.73
31	15.1	1.69	0.76
32	18.0	1.59	0.75
33	16.3	1.92	0.66
34	15.8	1.64	0.53
35	17.9	1.44	0.25
36	19.2	1.51	0.17
37	17.8	1.59	0.30
38	17.1	1.39	0.28
39	14.7	1.33	0.38
40	13.4	1.55	0.51
41	40.5	1.71	0.19
42	10.5	1.88	0.41
43	17.0	1.64	0.46
44	14.7	1.74	0.36
45	16.4	1.94	0.54
46	11.6	1.63	0.27
47	14.9	1.77	0.10
48	10.9	2.06	0.37
49	9.2	1.97	0.50
50	14.5	1.89	0.11
51	8.4	1.92	0.76
52	84.0	1.59	0.13
53	10.0	1.69	0.53
54	16.9	1.88	0.32
55	8.1	1.74	0.63
56	30.2	1.63	0.17

Table 1. Quality and quantity of RNA isolated from exosomes from serum was measured by NanoDrop.

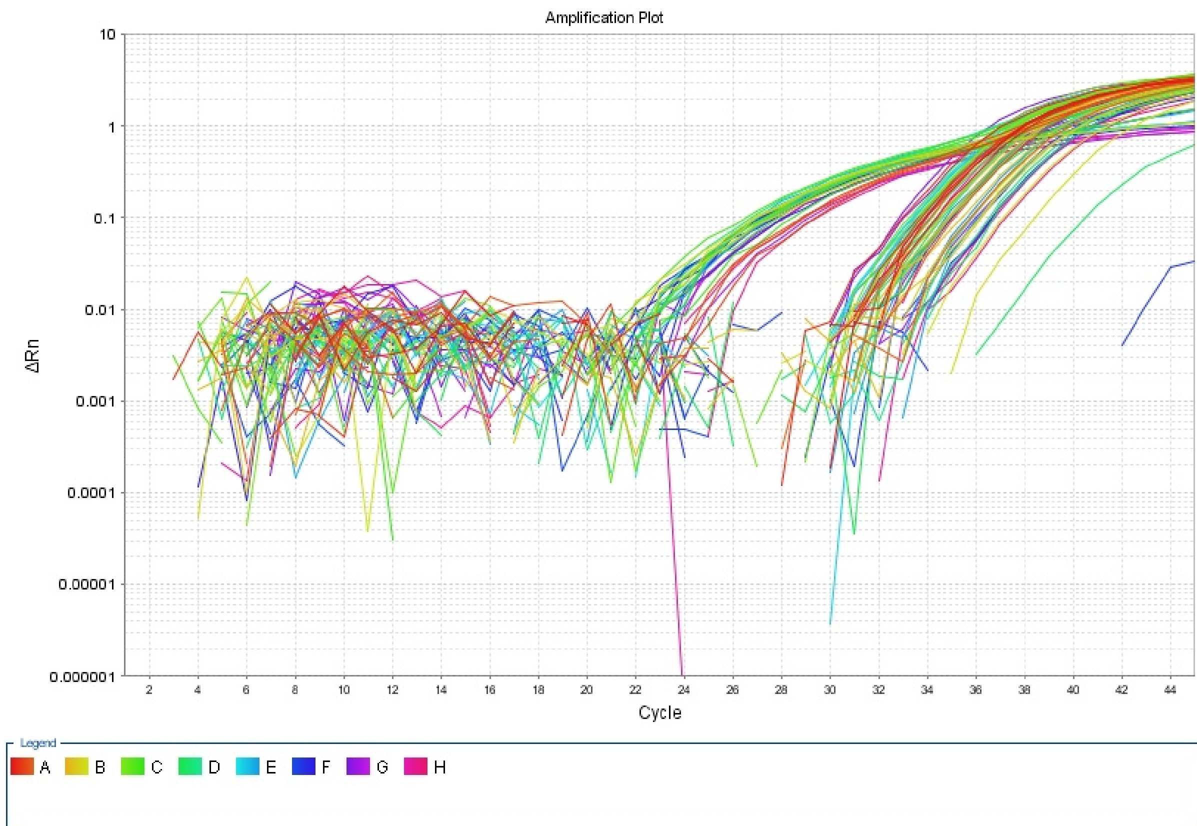


Figure 1. Amplification plot of qPCR ran for miR-125b and miR-155 with U6 snRNA as endogenous control.