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

Head of College Scholars List Scheme

Summer Studentship 2019

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: maureen.bain@glasgow.ac.uk **within four weeks of the end of the studentship**.

1. Student

   Surname: Purcell Forename: Alison

   E-mail address: 9405393p@student.gla.ac.uk

2. Supervisor:

   Surname: Kirschner Forename: Kristina

   E-mail address: kristina.kirschner@glasgow.ac.uk

3. Research Project Report

   3.1 Project Title (maximum 20 words): Inducing DNA damage to IMR90 fibroblasts to induce primary senescence via RasG12V activation, and quantification of resultant senescent cells.

   3.2 Project Lay Summary (copied from application):

   Accumulation of aged cells leads to impairment of organ function and is one of the biggest factors underlying for cancer and age-related diseases, such as dementia, in the elderly. In this project, I aim to characterise different types of ageing that occur in cells, a process called senescence. Cellular senescence can occur due to a variety of reasons,

such as damage to a cell, the activation of a cancer-causing gene. Senescence stops a cancer in the early stages, leading to elimination of pre-cancerous cells by the immune system. When senescent cells fail to be cleared, they can help cancer growth.

3.3 Start Date: 05/06/2019 Finish Date: 31/07/2019

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

To induce primary senescence by DNA damage in vitro and then stain for senescence markers of cell-cycle arrest and PAI-1. To then quantify the results using fluorescence microscopy, in an attempt to verify the use of Etoposide to induce primary senescence, and to optimise the experimental protocols.

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

DNA damage was induced in cultured IMR-90 cells by incubation with Etoposide for 48 - 62 hours to cause double strand DNA breaks. The cells were then stored at 37^{o}C in a low-oxygen incubator, and harvested at 4 different time points following the Etoposide incubation (days 4, 6, 11 and 13) for incubation and labelling with the modified thymidine analogue EdU. EdU is incorporated into DNA during cell cycle DNA synthesis and so is only present in proliferating cells (cell-cycle arrest is a feature of senescence and so no EdU should be present in senescent cells). The incorporated EdU was then fluorescently labelled (shown in green in figure 1 below) using the Click-iT cell proliferation imaging kit, to enable identification of dividing cells and allow for quantification of cell replication rates. The experiment was replicated 3 times, with a week between each starting point. The cells were additionally stained with DAPI (shown in blue in figure 1 below) to allow for total cell counts. A further primary/secondary

antibody test staining for the presence of the senescence marker PAI-1 was carried out. Cells were quantified by using fluorescence microscopy to photograph the slides and allow counting of the DAPI and EdU stained cells. Training in the following protocols was involved: splitting IMR90 cells, mixing IMR90 medium, Etoposide incubation, cell attachment to coverslips, EdU incubation, EdU detection, fixing cells, mounting coverslips onto microscope slides, and PAI-1 primary and secondary antibody staining. In addition, training was given on using the fluorescence microscope to visualise and capture cell images, and in quantification of the EdU incorporated cells.

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

The results were obtained from average counts of the percentage of EdU incorporating cells versus the total cell counts (DAPI stained) taken from multiple slides with an approximate cell count for each time period of n=300. The results indicate that incubating the IMR90 cells with Etoposide significantly reduced the number of observable replicating cells (shown in green in appendix 1, figures 1 and 2) compared with the control group of growing (untreated) cells, and therefore may indicate a significantly increase in cell senescence in the Etoposide treated group. The test staining for PAI-1 yielded no observable results.

3.7 Discussion (500 words max):

It has previously been shown that DNA damage can trigger cellular senescence (Chen et al., 2007) and that this is thought to be a key driver in ageing and cancer growth. The experiment outlined above sought to show that cell senescence could be achieved by inducing DNA damage by incubation with Etoposide, and that this could be measured by using EdU incorporation as a method of identifying the number of replicating cells in the

treated versus the untreated group (as cell-cycle arrest is a marker of senescence). The experiment was repeated 3 times with similar results and appears to show that using Etoposide to induce DNA damage does significantly reduce the number of replicating cells and that EdU incubation can be used successfully as a method of identifying, and therefore quantifying, replicating cells. That the Etoposide treated cells had stopped dividing was indicated by the severe reduction, or absence of EdU incorporated cells, as well as a greatly reduced cell count overall, compared with the control group (appendix 1, figures 3 and 4). However, whether the Etoposide treated cells had actually become senescent, was not proven as the test for an additional senescence marker, PAI-1, failed to yield any observable results – with no fluorescence visible, suggesting that the assay was unsuccessful. As a result, we can only infer that the treated cells had stopped dividing, but not that they had become senescent, as quiescent cells also exhibit cell cycle arrest. The reason for the failure of the PAI-1 assay is unknown, but perhaps there was a failure to accurately follow the protocol, or an incorrect secondary antibody was used. If more time was available the PAI-1 staining could be repeated, in order to detect the senescence marker and/or assays could also be done for other senescence markers such as SA-β-gal activity, in order to further strengthen the assertion of the presence of senescent cells.

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

   I found the experience extremely enjoyable and feel I have gained a large amount of practical experience and knowledge. The term-time lab experience we have had so far was limited and so this was a great opportunity to spend significant time working with cells and following experimental protocols through to complete an experiment. In addition, the experiment was repeated 3 times and so by the 3rd iteration I was able to work completely independently. This has given me a huge boost in my confidence in my practical skills and

will be a great help in the coming term-time labs. I was also given the opportunity to attend multiple presentations that are a frequent feature at the Beatson, and so I got additional exposure to other research projects that are ongoing at present. The lab team were incredibly friendly and supportive and made me feel welcome, and I feel the whole experience was invaluable both in terms of increasing my practical knowledge and skills, but also in giving me real experience of a working lab environment. I would definitely recommend this to any student wanting to pursue a career in lab-based research.

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

Not applicable yet as this is just the start of a new project in the lab

6. Signatures:

Supervisor	Date	Student	Date
	29/08/19		28/08/19

Appendix 1

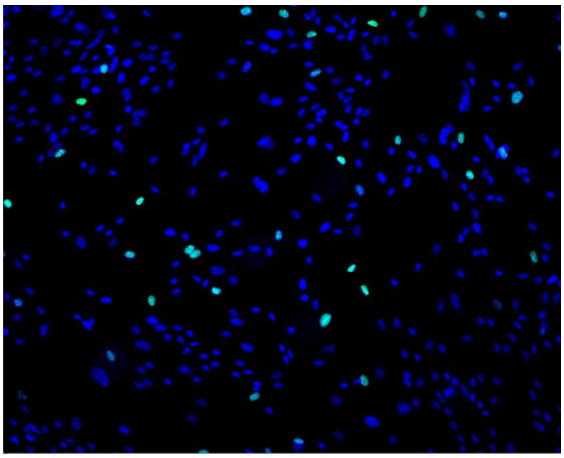

Figure 1. Fluorescence microscopy image of IMR90 cells with EdU incorporated cells stained in green and DAPI stained cells in blue. This is a sample from day 13 of the control group.

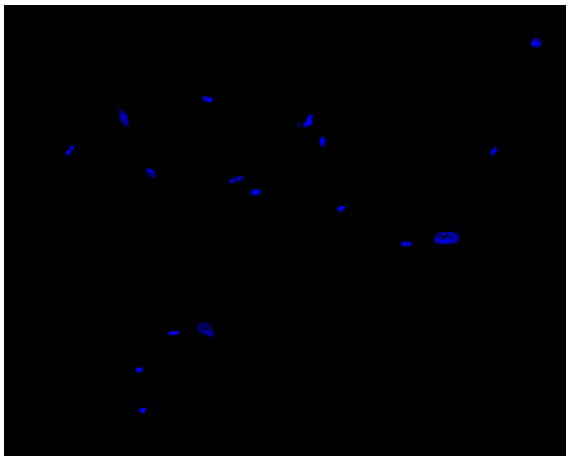

Figure 2. Fluorescence microscopy image of IMR90 cells with EdU incorporated cells stained in green and DAPI stained cells in blue. This is a sample from day 13 of the Etoposide treated group.

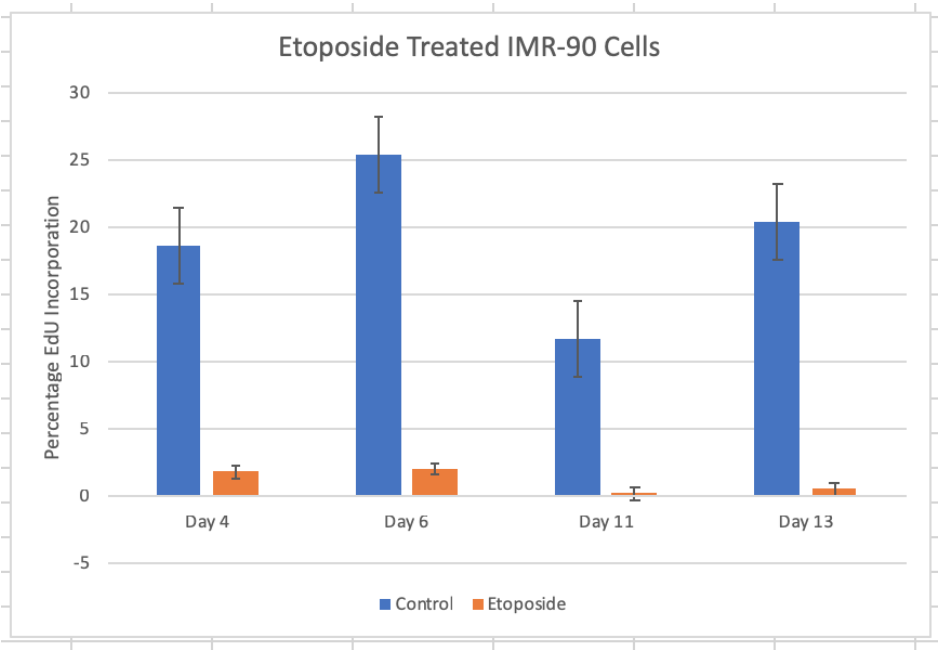

Figure 3. Graph of average percentage EdU Incorporation versus total cell counts for all 3 experimental runs. The timepoints day 4, day 6, day 11 and day 13 represent the days that EdU incubation was carried out. The counts are average percentages of EdU incorporated cells versus total cell counts (DAPI stained) taken from multiple slides with an approximate cell count of n=300. Average counts can be seen in tables 1 and 2 below.

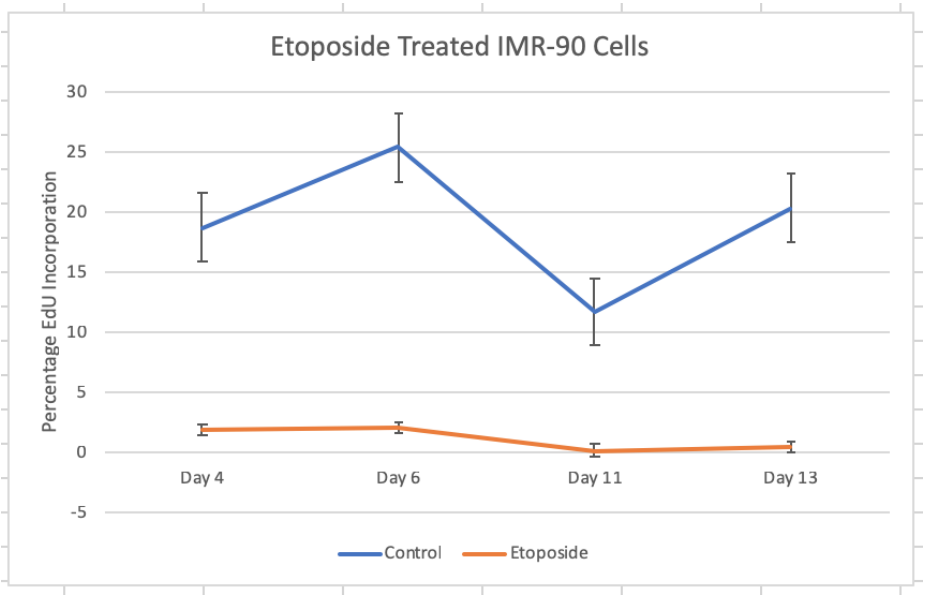

Figure 4. Graph of average percentage EdU Incorporation versus total cell counts for all 3 experimental runs. The timepoints day 4, day 6, day 11 and day 13 represent the days that EdU incubation was carried out. The counts are average percentages of EdU incorporated cells versus total cell counts (DAPI stained) taken from multiple slides with an approximate cell count of n=300. Average counts can be seen in tables 1 and 2 below.

Control IMR90 cells

	Day 4	Day 6	Day 11	Day 13
Batch #1 (% EdU incorporation)	N/A	22.54	9.94	6.8
Batch #2 (% EdU incorporation)	23.03	20.06	13.42	32.59
Batch #3 (% EdU incorporation)	14.24	33.33	11.63	21.41
Average	18.64	25.31	11.66	20.27

Table 1. Average cells counts for control cells over 3 runs (batches) of the experiment. Each batch represents a separate incidence of the experiment running through a period of 13 days. The timepoints day 4, day 6, day 11 and day 13 represent the days that EdU incubation was carried out. The counts are average percentages of EdU incorporated cells versus total cell counts (DAPI stained) taken from multiple slides with an approximate cell count of n=300.

Etoposide treated IMR90 cells

	Day 4	Day 6	Day 11	Day 13
Batch #1 (% EdU incorporation)	N/A	4.23	0	0
Batch #2 (% EdU incorporation)	1.6	1.65	0.29	1.32
Batch #3 (% EdU incorporation)	1.94	0	0	0
Average	1.77	1.96	0.1	0.44

Table 2. Average cells counts for Etoposide treated cells over 3 runs (batches) of the experiment. Each batch represents a separate incidence of the experiment running through a period of 13 days. The timepoints day 4, day 6, day 11 and day 13 represent the day following Etoposide incubation that EdU incubation was carried out. The counts are average percentages of EdU incorporated cells versus total cell counts (DAPI stained) taken from multiple slides with an approximate cell count of n=300.

References

Vaughan, D.E., Rai, R., Khan, S.S., Eren, M. and Ghosh, A.K., 2017. Plasminogen activator inhibitor-1 is a marker and a mediator of senescence. *Arteriosclerosis, thrombosis, and vascular biology*, *37*(8), pp.1446-1452.

Chen, J.H., Hales, C.N. and Ozanne, S.E., 2007. DNA damage, cellular senescence and organismal ageing: causal or correlative? *Nucleic acids research*, *35*(22), pp.7417-7428.