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**: Julius** Forename: **Shannen**

   E-mail address: **1002213j@student.gla.ac.uk**

2. Supervisor:

   Surname: **Roseweir** Forename: **Antonia**

   E-mail address: **antonia.roseweir@glasgow.ac.uk**

3. Research Project Report

   3.1 Project Title (maximum 20 words):

   The role of Src kinase in renal cancer, and the effect of Src kinase inhibition on non-metastaic renal carcinoma

   3.2 Project Lay Summary (copied from application):

   Around 9,300 people are diagnosed with renal cancer each year. Current treatment options include biological therapy with tyrosine kinase inhibitors (TKIs), which act by blocking enzymes involved in the signalling pathway which causes cancer cell growth.

   Src kinase is an enzyme (a non-receptor tyrosine kinase), which has been identified as the target of the TKIs dasatinib and saracatinib. My project will aim to determine the specific changes in behavior of renal cancer cells caused by Src kinase inhibition, by what processes

they are achieved, and if Src kinase inhibition is in fact the mechanism by which these drugs elicit there affects.

3.3 Start Date: **Monday 17th June 2013** Finish Date: **Friday 9th August 2013**

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

Our original aims were to investigate the effect of Src inhibitors in a non-metastatic renal carcinoma cell line, 769-P to compare if the effects seen in the 786-O cell line can be confirmed in cells from a separate renal cancer patient. To do this we planned to use two Src inhibitors, dasatinib and saracatinib, to assess the effects of inhibition on cellular proliferation, apoptosis, and motility and assess if the inhibitors are in fact acting via Src kinase. As all the experiments involving the 786-0s were not completed before the start of my project, rather than looking at the 769-Ps the aims of the project were changed to look at the effect of the Src kinase silencing on 786-0s.

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

1. **siRNA was used to remove all Src kinase from the 786-0 cell line, this was done using chemical to transfection with lipofectamine RNAiMAX for 72hours. Knockdown was confirmed by western blot.**
2. **To assess if the siRNA and inhibitors were active in the 786-0 cell line we looked at whether Src kinase activation via phosphorylation of Y416 or activation of the downstream marker FAK at Y861, is inhibited with increasing concentrations of inhibitor or with Src silencing. This was done via western blotting.**
3. **Silenced cells were then used to assess the effects of the knockdown on functional outputs (apoptosis and proliferation) in conjunction with Src inhibitors to establish if dasatinib or saracatinib are working via Src kinase inhibition. Proliferation was determined directly by cleavage of wst-1 reagent. Apoptosis was measured by Cell Death Detection ELISA PLUS allowing the quantification of histone complexed DNA fragments.**

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

1. **Firstly, we showed that whilst dasatinib and saracatinib both inhibit the activation of the downstream marker FAK at Y861, only dasatinib inhibited phosphorylation of the Src (Y416) site. This was shown by the western blots we ran with 786-O cells treated with both of the drugs.**
2. **We showed that the silencing we performed had worked and that we had successfully removed all of the Src kinase from the cells. This was seen in the western blots we ran of the 786-O cells protein via the western blots for Src (Y416), FAK (Y861) and total Src.**

3. **The proliferation assays looked at the amount of cell proliferation seen when the cells are treated with different concentrations of either dasatinib or saractinib. The assays showed that dasatinib caused a decrease in proliferation in the cells that had Src kinase but had little effect on those that had been silenced. Saracatinib on the other hand had little effect on proliferation regardless of whether the cells where silenced or not. However, this needs to be repeated to confirm this result.**
4. **In the apoptosis assays we saw that only dasatinib increased apoptosis and that this appears to be independent of Src and silencing had no effect. However, this needs to be repeated to confirm these results.**

3.7 Discussion (500 words max):

Renal cancer is the eighth most common cancer in adults in the UK. The most common type is known as renal cell carcinoma, which accounts for more than 80% of all kidney cancers. The treatment of kidney cancer depends on the size and spread of the cancer. Most commonly, surgery is the first course of action, with the aim of removing the cancer cells. Unlike most other cancers, chemotherapy is not very effective in treating kidney cancer, but as I discussed above there are now non-surgical treatments available, such as radiotherapy or targeted drug therapies but these are showing little effect if the cancer is aggressive. Therefore we need new targeted therapies and this project focused on one such therapeutic, Src kinase inhibitors.

Our proliferation assays investigated the level of cell proliferation seen when 786-O renal carcinoma cells are treated with different concentrations of two Src inhibitors, dasatinib and saracatinib. This was performed using both non-targeting and Src silenced cells, to compare the effect of the inhibitors in the presence and absence of Src kinase. The assays showed that dasatinib caused a decrease in proliferation in the cell when Src kinase was present but had little effect on those that had been silenced, indicating that dasatinib does work via Src. Saracatinib on the other hand had little effect on proliferation regardless of whether the cells where silenced or not suggesting that saracatinib doesn't affect proliferation or work via Src kinase and must work through another family member.

The apoptosis assays showed that dasatinib can increase apoptosis in 786-O cells and that this was regardless of Src kinase as Src siRNA had no effect on this increase. The difference seen between apoptosis and proliferation may be due to another family member compensating for the loss of Src kinase in the apoptosis pathway but not the proliferation cascade. Whereas, Saracatinib had no effect on apoptosis, similar to the results seen for proliferation, again possibly due to working on another protein in the pathway.

The results suggest that dasatinib and saracatinib elicit there affects through different pathways and that while saracatinib is an effective drug against a number of cancers it doesn't work by decreasing cancer cell growth or increasing cell death. However, others within the lab have shown that saracatinib significantly decreases migration and therefore

may work by decreasing metastasis. Unfortunately, due to an infection in the cells, time did not permit me to carry out the intended wound healing experiments. Although our findings suggest that saracatinib doesn't work via the Src kinase pathway the western blots did suggest saracatinib does cause a decrease in the pathways downstream marker FAK (Y861). This implies that saracatinib may work by affecting the pathway at a different point further down the cascade or via a different Src family kinase. However, we have confirmed that Dasatinib does target Src kinase to elicit effects on apoptosis and proliferation.

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

My time spent with the University's Institute of Cancer Sciences team has been thoroughly enjoyed. Before embarking on my project I was extremely excited about the opportunity to see the inner working of a lab and eager to get involved in some scientific research and I can honestly say that the experience did not disappoint. During this placement I've gotten to take part in multiple practical experiments, been trained in many of the routinely used cancer lab techniques and received teaching in data analysis and interpretation. I also got the take part in a lot of independent work and once trained was treated not like a student but like a member of the team. Unfortunately midway through the project the cells I was working with contracted an infection and had to be disposed of. This caused us to fall behind and so we decided to focus on assessing the cells proliferation and apoptosis and leave out testing motility. This was disappointing however I did get to see the labs infection control procedures in practise and it gave me a realistic insight into the highs and lows of working in a lab.

One of the more surprising things I've learnt from this placement is how temperamental science can be. Sometimes experiments fail for a reason and sometimes experiments fail for no reason, things that work perfectly for weeks can suddenly stop working, offering no explanation for the change. While this was disheartening the satisfaction felt from achieving a good result made it all worthwhile.

This project has been an amazing opportunity, It's given me first-hand experience of what life in research is like, something that will be prove invaluable when it comes to deciding on academic and research posts in the future.

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

This project will contribute to a larger body of work that is due to be published by m supervisor and her team at the University's Institute for Cancer Sciences.

6. Signatures: Supervisor Date Student Date

09/08/2013

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