

The Urology Foundation Annual Report For Funding

Dr Simon Crabb, Associate Professor in Medical Oncology, University of Southampton

1.) Title of Project

Investigating interactions between vacuolar ATPase proton pumps and androgen receptor signalling in prostate cancer

2.) Starting date and Completion date

Bradleigh Whitton was appointed as a PhD student to undertake this laboratory based doctoral research project over four years with funding provided jointly through The Urology Foundation and Wessex Medical Research. He commenced this in October 2015 and is working jointly between the laboratories of Dr Simon Crabb, Cancer Sciences Unit, and Dr Haruko Okamoto, Centre for Biological Sciences, at the University of Southampton. His supervisory team is completed by Professor Graham Packham within the Cancer Sciences Unit.

3.) Date of Report

This is our first annual report, completed 18th August 2016.

4.) Research hypothesis

Our original proposal was to look at an aspect of prostate cancer biology which has not received prior attention and which might hold potential for therapeutic exploitation. It is well established that the key driver in the development and progression of prostate cancer is through 'androgen receptors' which are stimulated by male hormones including testosterone. This forms the basis of hormonal therapy that is a critical component of how we treat this disease. The androgen receptor is also critical in mechanisms responsible for resistance of prostate cancers to hormonal therapy by development of structural mutations. Within our supervisory team, Dr Okamoto has expertise in the biology of a group of proteins that form 'cellular pumps' called vacuolar ATPases. These are important in maintaining cellular homeostasis and in particular in maintaining a healthy acid/base balance within cells. There is some evidence to suggest that V-ATPases might be important in development of some cancers. In this project we were interested to test the hypothesis that: **V-ATPases would activate and drive activity of the androgen receptor in prostate cancer**. If so then they might be relevant potentially to the use of hormonal therapy and also might represent a therapeutic target in their own right.

5.) Study plan

This is a pre-clinical cell and molecular biology project based jointly in the Crabb and Okamoto laboratories. We have taken two parallel approaches to the first year of work which are outlined below.

6.) Key Findings

Firstly we have undertaken work in prostate cancer cell lines to test the potential interaction between the androgen receptor and V-ATPases. This work involved depleting V-ATPase function with specific chemical inhibitors and then testing the activity of the androgen receptor. This was undertaken by measuring the level of both the protein and RNA message of target genes for the androgen receptor.

(This mirrors the approach to measuring prostate cancer activity in the clinic through levels of a protein called 'prostate specific antigen'). We have been able to establish during this first year that reduction in V-ATPase activity does indeed reduce the activity of the androgen receptor. This is of significant importance as it establishes a novel mechanism of androgen receptor function and confirms the basis for this project. The detail of this molecular interaction will now form the basis of a substantial part of the next 3 years of work for this student. We will undertake experiments in which we will artificially deplete the various components of the V-ATPase protein complex and also dissect the mechanics of its interaction with the androgen receptor. We will also test experimental models of hormonal therapy resistance established in the Crabb laboratory to determine the potential relevance of V-ATPases in this important clinical setting.

In addition a major second strand of work is to develop understanding of abnormalities in the structure of subunits of the V-ATPase protein complex that may be relevant in cancer. It is known that some cancers, potentially including prostate cancer, exhibit abnormal forms of V-ATPase subunits that may increase or decrease its function and therefore impact on cancer behaviour. To investigate this further our student has made 'clones' of V-ATPases into which he has inserted mutations of the basic DNA code. This will allow us to undertake experiments in which we can control the presence of normal or abnormal forms of V-ATPases. We will then plan to insert these into prostate cancer cells in the laboratory and test the impact that it has. The first part of this process which is construction of the mutant forms has been completed and we will spend time in the next year undertaking experiments with these. We will do this in prostate cancer models but the work has potential relevance in other cancers also.

7.) Student progress

Bradleigh Whitton has been appointed as the doctoral student for this project. He was required to complete a report at 5 months which was very satisfactory and required almost no alterations. The next assessment point is an 11 month report which is in progress at the time of writing. He will then undertake a 'Transfer Viva' in the first quarter of 2017. For this he is required to produce a thesis outlining work to date and future plans. This is examined by a senior member of the University from a relevant discipline who is not directly involved with the project. In addition Bradleigh is drafting a review paper to cover the potential role of V-ATPases in cancer which we intend to submit for publication, most likely to the British Journal of Cancer. More generally Bradleigh has integrated well into our laboratory groups and mastered experimental techniques in a very satisfactory manner. We are very pleased with his ability to work at an appropriate level of independence and to drive the project forward.

8.) Problems encountered (if any)

There have not been any particular problems or complications so far and essentially the project is running to plan at this stage.

9.) Do you wish to apply for further TUF funding to continue this study?

The first year of this project has proceeded in a highly satisfactory manner. We have achieved the aims for this stage and in particular have established a link at a molecular level between inhibition of V-ATPase activity and androgen receptor function in prostate cancer cells. The state of progress is

therefore promising and results to date support the remaining three years of this project. In addition, as a PhD project, we are very pleased with the progress of our student. He has settled into the project very well and shows every sign of being able to develop a set of data that should be suitable for successful examination by the end of the project. Thus, in addition to producing novel data on prostate cancer biology we anticipate training of this student to a level doctoral level. As such we are enthusiastically requesting ongoing funding from The Urology Foundation according to the original award agreement and in collaboration also with Wessex Medical Research.

Dr SJ Crabb

August 2016