FINAL REPORT TO THE RACP FOUNDATION

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Name	Jun Yang	
Award Received	7/1/2015	
Report Date	16/6/15	
Project Title	Identification of coregulator proteins that interact with the mineralocorticoid receptor to selectively modulate receptor function in cardiovascular disease	
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Funding Period	**Start:** **7/1/15**	**Finish:** 7/12/15

Lay Summary: *Please provide a brief plain English summary suitable for media release.*	My research seeks to understand how the mineralocorticoid receptor (MR) functions in the heart compared to the kidneys. The MR in the kidneys binds the hormone aldosterone which controls salt and water balance in the body. However, the MR in the heart, when activated, has been shown to increase inflammation and heart failure. My studies have identified novel proteins which affect the activity of the MR differentially in the heart compared to the kidneys. It may allow the design of new drugs to block MR function only in the heart, without affecting salt balance in the kidneys, and therefore treat heart failure with minimal side effects.

Project Aims/ Objectives: *Please state the aims and objectives and how they were achieved.*	My research aims were to: 1. Use T7 phage display to identify novel, functionally important MR-coregulator interactions; 2. Demonstrate cell-specific differences in the interaction between the MR and the novel proteins identified in aim (1) which may be potential targets for cardiovascular drug discovery; 3. Examine the role of MR-coregulators in macrophages. I have identified two novel coregulator molecules, Gemin4 and Timeless, which appear to differentially affect MR activity in kidney vs heart cell lines. They have not yet been examined in macrophage cell lines as macrophages are difficult to manipulate in vitro and optimisation is being performed.

Research conducted to date: *Please provide a brief summary of methodology, trials, experimental procedures, etc.*	T7 phage display could not be performed due to the technical challenge of creating a macrophage cDNA library. However, results from earlier studies led to the identification of two novel proteins, Gemin4 and Timeless, for further characterization. The interaction of both proteins with the MR were tested using MR-responsive reporter genes in transactivation assays in renal- and heart-derived cell lines. The proteins were then over-expressed by transfection or knocked down using siRNA in cells to study their effect on endogenous MR-dependent gene expression. In the case of Gemin4, this was followed by immunofluorescence co-localisation to confirm its interaction with the MR in the cell nucleus. Co-immunoprecipitation was performed for Timeless and the MR to confirm their physical interaction. The ligand-specificity of both proteins were also examined by transfecting them with a range of other steroid hormone receptors. The plan is to repeat the above experiments in a macrophage-derived cell line to determine the tissue-selectivity of both proteins. However, optimisation is still being performed due to the difficulty of transfecting macrophages.

Significance and Outcomes: *Please state significance, for your field and medicine in general, and outcomes of the research project.* *Please list any proposed future research in this field*	A review of the current literature suggests that a significant part of selective nuclear receptor modulator pharmacodynamics is due to the unique conformational changes they induce within the receptor with subsequent differential coregulator recruitment. A successful example of a selective modulator is Tamoxifen which is commonly used in the treatment of breast cancer to block estrogen receptor in the breast but not in the bones. In the research setting, other small molecule nuclear receptor-coregulator inhibitors have been discovered for the estrogen, androgen, thyroid hormone and vitamin D receptors using structure-inspired rational design and high throughput screening. These studies highlight the importance of understanding the coregulator profile of a nuclear receptor, which is what our study aimed to achieve for the MR. The identification of two tissue-selective coregulators is a significant contribution to the field fo MR biology given the dearth of existing data on coregulators. It opens up the exciting possibility of designing therapeutic agents that target MR activity in a tissue-selective manner so as to better separate desirable therapeutic efficacy (ie. reduce cardiac inflammation and fibrosis) from undesirable adverse effects (ie. hyperkalemia due to renal MR blockade). This represents an innovative approach to rational drug design for the treatment of cardiac fibrosis and heart failure. The benefits of MR antagonists may extend to other diseases with a significant component of tissue inflammation such as the metabolic syndrome. Further studies can explore: - the effect of MR antagonists on adipose tissue and insulin sensitivity - MR coregulators within adipocytes and how they can be selectively targeted - biomarkers of MR antagonist efficacy in heart failure treatment

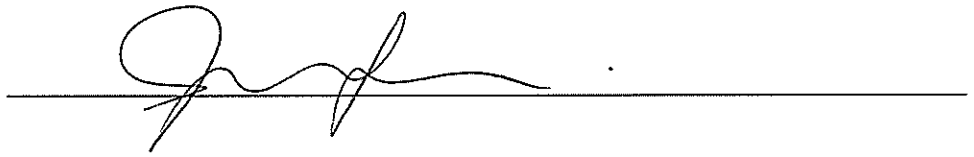
Academic Output: *Publications and/or abstracts produced as a result of the project.*	1) Yang J, Fuller PJ, Morgan J, Shibata H, Clyne CD, Young MJ (2015) Gemin4 functions as a coregulator of the mineralocorticoid receptor. Journal of Molecular Endocrinology, 2015 Apr;54(2):149-60 2) Yang J, Fuller PJ, Morgan J, Shibata H, McDonnell DP, Clyne CD, Young MJ (2014) Use of phage display to identify novel mineralocorticoid receptor-interacting proteins. Molecular Endocrinology, 28(9):1571-84 3) Fuller PJ, Yang J and Young MJ (2015) Corticosteroid receptors. In: Nuclear Receptors: From Structure to the Clinic, Iain J. McEwan IJ and Kumar R (Editors) Springer Heidelberg. (book chapter)

Additional Advice and Comments *Please state items of interest which have arisen as a result of the project, such presentations or other outcomes.*	Since receiving the RACP Foundation for High Blood Presssure Research Award in 2014, I have presented my work at one national meeting, published two journal articles, writen and contributed to two book chapters and plan to present new work at another national (Endocrine Society of Australia) meeting in August 2015 in additional to an international ADMIRE (Aldosterone and Mineralcorticoid Receptor) Conference in Zermatt in October 2015.

Acknowledgements	I am grateful for my mentors, Dr Morag Young and Prof Peter Fuller, for their ongoing support of my work in the Cardiovascular Endocrinology Laboratory. I also thank Mr James Morgan for assisting me in the lab by sharing experimental work.

Award Recipient Signature:

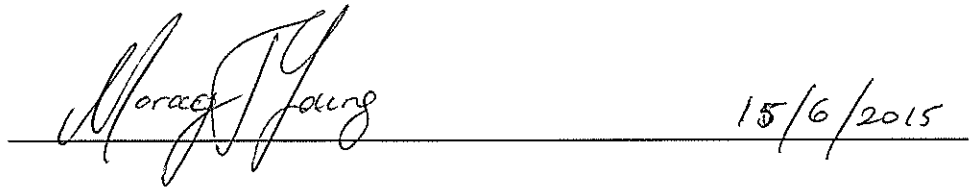
I certify that the information supplied in this report is true and correct. I understand that the Royal Australasian College of Physicians may wish to verify this information with any institution or individual. I consent to such inquiries.

Signature: 

Chief Investigator/Supervisor Signature:

I, Morag Young **of the** Hudson Institute of Medical Research
(Supervisor) *(Name of institution where research was undertaken)*

have read this report and believe it to be true and correct version of the research undertaken during this period.

Signature:  15/6/2015