CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF MUTANT p53 SECRETOME IN HUMAN CANCER

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Bachelor of Science in Biotechnology (Hons)

Thesis submitted for the degree of

Doctor of Philosophy

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June 2016
Dedication

This thesis is dedicated to my loving mom and dad
Usha and Purushottam man Shakya,
my sisters Punam, Kalyani and Merina Shakya
and my partner Aabhash Shrestha
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ABSTRACT

Among several genetic alterations in human cancer, mutations in the TP53 tumour suppressor gene represent the most common, occurring in approximately 50% of all human cancers. The majority of these mutations in p53 are missense mutations, resulting in cancer cells expressing stable, full-length mutated p53 proteins. Missense mutant p53’s exhibit loss of tumour suppressive property of wild-type p53, dominant negative effects that can inactivate any wild-type p53 protein, and gain-of-function (GOF) properties that promote tumour progression and metastasis. Evidence suggests that cancer cells depend on the sustained expression of mutant p53 GOF. Thus, identifying the common downstream factor that drive mutant p53 GOF can provide an attractive approach to therapeutically target mutant p53 expressing tumours.

This thesis presents the study of the characterization and functional analysis of mutant p53 secreted factors called “the mutant p53 secretome”. In particular, the thesis aims at identifying the critical secreted effector of mutant p53 GOF that can serve as a potential therapeutic target for treatment of mutant p53 expressing tumours. Furthermore, the thesis investigates the association of the identified factor within the secretome with clinical parameters such as patient’s survival. This thesis makes several original contributions to the field of cancer research, which are briefed below.

Firstly, the mutant p53 induced secretome was characterized using quantitative proteomics of conditioned medium from mutant p53 expressing inducible H1299 human lung cancer
cells. The majority of the identified secreted proteins were the transcriptional targets influenced by mutant p53. Alpha-1 antitrypsin (A1AT) was selected for further investigation, as it was the protein showing the highest expression in the mutant p53 secretome.

The role of A1AT in driving the oncogenic activity of mutant p53 in human lung cancer cells was explored. A1AT was shown to drive mutant p53 induced invasion in lung cancer cell lines. Ablation of A1AT using antibodies and gene knockdown approaches inhibited the mutant p53 driven invasion, providing a rational to investigate the development of antibody-based cancer therapies that target A1AT.

The clinical association of A1AT was further investigated in tissue microarray (TMA) samples of lung adenocarcinoma (ADC) patients. Mutant p53 expression was shown to correlate with A1AT, which validates in vivo that A1AT is a bonafide target of mutant p53. Furthermore, elevated expression of A1AT was demonstrated to correlate with increased local invasion and poor prognosis of lung ADC patients.

Mutant p53 is reported to function as an aberrant transcription factor that can interact with other transcription factors to reprogram the cellular transcriptome of cancer cells. The mechanism of regulation of A1AT by mutant p53 was confirmed to involve p63.
The role of A1AT in driving the mutant p53 induced invasive behavior of breast cancer cells was also explored, and a relationship of A1AT with p53 status and with different subtypes of breast cancer was established. In p53 mutant basal-like subtypes, A1AT expression was shown to drive invasion and treatment with anti-A1AT antibodies inhibited invasion. This suggests that the A1AT-targeted are potential therapies in various cancer types and its regulation in breast cancer may also extend beyond p53.

Collectively, these studies provide new insights into the invasive behavior of mutant p53 that are manifested through aberrant secretion of extracellular proteins. The identification of A1AT as a critical and indispensable effector of mutant p53 gain-of-function offers a new therapeutic options for treatment of p53 mutant tumours. The findings in this thesis involve significant elements of novelty describing how mutant p53 influences the cellular secretome.
DECLARATION

I, Reshma Shakya certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify no part of his thesis will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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AWARDS & SCHOLARSHIPS

SAHMRI Beat Cancer Travel Grant 2015

University of Adelaide, Discipline of Medicine Travel Grant 2015

Walter & Dorothy Duncan (for Research & Travel) 2015

Best Poster Presentation (School of Medicine Award) 2015
University of Adelaide, Postgraduate Research Conference

Royal Adelaide Hospital Research Foundation 2013
Dawes Top-Up Scholarship

Australian Post Graduate Scholarship 2012
PUBLICATIONS


Some relevant component of the work has been presented in following national and international conferences and seminars:


ACKNOWLEDGEMENTS

First and foremost, I would like to convey my deepest gratitude to my supervisors Prof. David Frederick Callen, Dr. Paul Matthew Neilsen and Dr. Gerard Tarulli for their guidance and support throughout my candidature. My principal supervisor, Prof. David Callen advised me with his open view and broad knowledge in the field of genetics and cancer biology. His thoughtful comments were always constructive and fruitful to improve the quality of my research. I am also grateful to him for providing me opportunity to conduct PhD project in his laboratory and for providing invaluable support and guidance to help me grow as a researcher. In addition, my external supervisor Dr. Paul Matthew Neilsen provided me with his solid knowledge in the field of mutant p53 in cancer biology. He served as my principal supervisor for one and half year during the candidature. He has significantly helped me to embark in the field of mutant p53 and find my direction in exploring the global influence of mutant p53 secretome in cancer. Further, I would like to express my gratitude to my co-supervisor, Dr. Gerard Tarulli for providing support, encouragement and advice, when it counted most. Dr. Tarulli’s expertise in the field of confocal microscopy and digital image analysis was of great importance towards my PhD research. He always encouraged me to conduct high quality research and publication. Besides his enthusiastic supervision, he has helped me to develop professionally and build my confidence in research.

Another key person whom I am strongly indebted to is my good friend and brilliant researcher, Dr. Kathleen Irene Pishas who provided continuous support and encouragement throughout the course of my PhD. In addition, my sincere gratitude goes to Dr. Noor Alia
Lokman, a real CAM assay expert, for her significant help towards using CAM assay models for my study. Noor was also of big help after the CAM assay was completed and tremendously helped me to process and analyze the results from assay. I am also indebted to Dr Carmela Ricciardeli, an expert in biostatistics for helping me to use SPSS program for the analysis of clinical data. I would also like to thank our collaborators in Sydney, Prof Wendy Cooper, Prof Maija RJ Kohonen Corish, and Christina I selinger for providing us with TMAs of lung ADC patient’s tumors samples which has formed a major part of my study. I would like to extend my gratefulness to all the patients who selflessly donated their tissues for research purpose.

I am indebted to Prof. Wayne Tilley and his group from Dame Roma Mitchell Cancer Research Laboratory (DRMCRL) for being kind and for providing space and reagents to conduct immunohistochemistry in their laboratory. I would also like to thank Prof Tilley, Dr Theresa Hickey, Dr Gerard Tarulli and Dr Luke Selth for inviting me to attend and present my research at their weekly group meeting. I would like to thank the office and support staff of the school of medicine, including IT officers Vanessa Burzacott, Christina Tsaousidis and Ryan King and administrative staff, Liz westwood, for her support and assistance. I would also like to thank Head of Discipline of Medicine, Prof. Gary Wittert and Head of School of Medicine, Prof Alastair Burt for their support with research travels and softwares required during my candidature. In addition, I am thankful to health and safety officer, Andrew Gyory who provided me an-depth practical training on health and safety. As part of my role as school’s health and safety student representative, I am thankful for the opportunity I received
to conduct several inspections in research laboratories including hospital based research centres.

My jouney de PhD is really memorable for me because of my great friends and colleagues in cancer therapeutics laboratory (CTL) and DRMCRL. My great friends, Alaknanda Adwal, Qingging wang, Sheng Lei, Feng yu and Dr Rajdeep Das were always there for me to discuss both scientific and non-scientific life issues together and laugh at our problems and difficulties as PhD students. I am also thankful to Dr Andrew Turner for supporting me and for reviewing and proofreading my papers and thesis. In addition, my sincere gratitude goes to past lab members, Renee Schulz and Rebecca Haycox for their technical support.

Looking back at my alma mater, I am indebted to my Honor’s Postgraduate Coordinator, Dr. Mukunda Ranjit for his great supervision and encouraging attitude towards research. Other scholars who contributed towards my academic backgrounds are Prof Don Cooper, Dr Praphul Shakya, Dr Pramod Aryal, Dr Manushree Hada, Dr Krishna Manandhar, Dr Tribikram bhattarai, Mr Ramesh Rajbanshi and Mr Manoj Chetri.

I recognize that this research would not have been possible without the financial assistance of Australian Government via a generous Australian Postgraduate Award (APA) Scholarship. During my candidature, I was awarded several other travel grants, scholarships and awards by several organizations. Here, I would like to deeply appreciate these organizations support including Dawes Top-Up Award from Royal Adelaide Hospital

All these generous travel support enabled me to attend and present my research findings at the Developmental biology and cancer conference that was organized by American Association for Cancer Research (AACR) in Boston, United states last year. In addition to presenting my research at conference, I received a great opportunity from Prof Rakesh K. Jain, a director of Edwin L.Steele Laboratory from tumor biology at Harvard University to visit his laboratory and discuss my research plans and interests. While in Steele lab, I met Professor Dan Duda and Dr Igor Garkavtsev from Harvard University. Prof Duda kindly introduced me to his interest on understanding the interaction of immune cells and stromal cells in chemo and radio resistance pancreatic adenocarcinoma. It was great to learn about most cutting edge field of cancer immunology and at the same time, discuss my own research interest in tumour microenvironment with these renowned researchers.

Back to my home country, my endless gratitude goes to my mother and father who always endow me with infinite support, encouragement, continuous love, well wishes and patience. I also thank my best and kindest sisters for their love, support and inspiration. Last but not the least, my heartfelt thanks are due to my partner, Aabhash who stood by me in all ups and downs of my PhD and always endowed me with his endless love and support.

-Reshma Shakya