Biomarkers of resistance to anti-*EGFR* in wild type *KRAS/BRAF* colorectal cancer cell lines

Thesis submitted for the degree of Doctor of Philosophy

Shalini Sree Kumar

Bachelor of Science (Honours), Master of Science (Medical Sciences)

School of Medical Sciences, Discipline of Physiology

University of Adelaide

January 2015

TABLE OF CONTENTS

Declaration

I 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 that no part of this work 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 the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Shalini Sree Kumar

ACKNOWLEDGMENTS

"Be not afraid of greatness. Some are born great, some achieve greatness, and others have greatness thrust upon them."

~ William Shakespeare

First and foremost, I would like to extend my deepest gratitude to my supervisor, Dr Jennifer Hardingham for giving me the opportunity to pursue this project and learn from her vast expertise in colorectal cancer research. I would not have been able to complete this journey without your unwavering support and guidance. Thank you for all those instances that has propelled me to reach greater heights in the realm of my work and research in general, and I truly hope I have lived up to your expectations.

I would like to express my gratitude to my co-supervisor Associate Professor Timothy Price whom I immensely respect. Your support, guidance and assistance were invaluable to me. I truly appreciate all of the help I received from proof-reading my manuscripts and thesis to giving me valuable insights to my research during my presentations and to assisting with securing the laboratory visits in Europe. Your thoughtful guidance is much appreciated.

Thank you Dr Joanne Bowen, Dr Amanda Townsend and Dr Christopher Hocking, your advice for this project and manuscripts were very valuable. My deepest appreciation also goes to the funding bodies of this project, the University of Adelaide and the Queen Elizabeth Hospital.

My biggest thanks and gratitude go to Joe Wrin and Dr Maressa Bruhn for creating a welcoming and friendly environment to work in and sharing their priceless insights into research methodologies and other approaches, as well as showing me how

to fully immerse in the laboratory experience. I will definitely think back fondly on our weekly extra hot curry catch-ups and exchanging musical favourites while we whiled away in the lab and I definitely would not have been able to finish curating the mountain that is the PhD thesis if it wasn't for those moments of sanity (or insanity as Joe would argue and I would agree). I would also like to express my deepest thanks to Aravind Shivasami, our former colleague who also gave me valuable guidance in a number of laboratory techniques and constantly encouraged me to keep going.

I would also like to express my gratitude to all of the friends I have made at the Basil Hetzel Institute (BHI), particularly Ranjani, Irene, Amanda and Amilia. Thank you all so much for your lovely company, great conversations and the much-needed laughter throughout my time at BHI. I will forever cherish and remember our times at BHI and the strong friendship that has extended beyond the lab. Thank you to Pallave, Danushka, Harsh, Zeya, Hilary, Caroline, Omar and Matt, all of whom in one way or the other have helped me, encouraged me and supported me throughout my time at BHI. I would also like to extend my gratitude to my friends outside BHI, near and far, who have always shown their unwavering support and been there for me whenever I needed them: Aathi, Radhi, Azizah, Kaminee, Lyana and Vivian. Thank you for everything!

Last but definitely not least I owe my deepest gratitude and appreciation to my family. Mom, Dad and my brother Prashanth, I wouldn't have made it through these three and a half years in a foreign land, in a home away home, without your annual visitations, unconditional love, constant encouragement, support and care. To also my extended my family in India: Amma, Uncle Viji, Aunty Sumathi, Santhosh, Reshmi and dearest Lohita, all of whom never failed to bring a smile to my face during our weekly phone calls, constantly supporting and encouraging me to stay strong and chase my dreams. In the words of the rapper Drake, *"Started from the bottom, now we're here."*

ABSTRACT

Colorectal cancer (CRC) is a leading cause of cancer death worldwide and despite significant improvement the median survival remains relatively poor. The use of targeted therapies like cetuximab and panitumumab inhibiting the epidermal growth factor receptor (*EGFR*) offer promise in improving patient outcomes. However, a high proportion of CRC patients show resistance to anti-*EGFR* therapy. Biomarkers such as mutant *KRAS* or *BRAF* predict resistance to anti-*EGFR* therapy in only a subset of patients and we hypothesise that other biomarkers for resistance to *EGFR* targeted therapies exist. The studies presented in this thesis aimed to determine other biomarkers of resistance to anti-*EGFR* therapy in wild type *KRAS* and *BRAF* CRC cell lines.

Following RT-Profiler Array analysis, the 3 most significantly upregulated genes amongst the 3 anti-*EGFR* resistant CRC cell lines (SNU-C1, SW48 and COLO-320DM) were chosen as candidate biomarkers of resistance: *HBEGF* (heparin-binding epidermal growth factor-like growth factor), *EGR1* (early growth response protein 1) and *AKT3* (protein kinase B gamma) were validated using qRT-PCR. *HBEGF* is a member of EGF-like growth factor family is a potent inducer of tumour growth, angiogenesis, and implicated in metastasis. *EGR1* is a transcription factor implicated in cell growth, survival, transformation, tumour progression. *AKT3* is a serine/threonine kinase and a downstream mediator of *PI3K-AKT-mTOR* pathway resulting in cell proliferation, cell survival and angiogenesis. *HBEGF* was knocked down by 79.4% in SNUC1, *EGR1* was knocked down by 85.6% in SW48 and *AKT3* was knocked down by 95.3% in COLO-320DM, as validated by qRT-PCR and western blot. Following knockdown, these cell lines were treated with anti-*EGFR*, and SNU-C1 had proliferation rate of 49.1% (83.8% before knockdown), SW48 yielded proliferation rate

of 46.9% (70% before knockdown) and COLO-320DM had proliferation rate of 64.1% (68.3% before knockdown). This suggests that the resistant phenotype of these cell lines was reversed. The expression of these markers was also elucidated using immunohistochemistry on mCRC primary tumour tissues from 10 patients that had undergone cetuximab monotherapy. Some 50% of these patients had overexpression of two or more of these markers, and these patients did not respond to cetuximab, suggesting that these overexpressed biomarkers might be involved in circumventing cetuximab to confer resistance.

One of the studies presented in this thesis also explored the *KRAS G13D* phenomenon and the effect of cetuximab and panitumumab on cell lines harbouring different mutational status. Previous clinical studies have demonstrated that a proportion of *KRAS G13D* harbouring tumour patients respond to the anti-*EGFR* therapies, and a large proportion of *KRAS* WT patients do not respond. After treatment with cetuximab or panitumumab, the *KRAS G13D* mutant cell lines showed intermediate sensitivity to both treatments, between the resistant *KRAS G12V* mutant cell line and the sensitive WT *KRAS* cell line. One of the *G13D* cell lines was significantly more sensitive to panitumumab than to cetuximab. This study demonstrated that specific *KRAS* mutation determines the responsiveness to anti-*EGFR* monoclonal antibody treatment, corresponding to previously reported clinical observations.

In conclusion, the studies presented in this thesis have demonstrated that components of *EGFR* signalling cascade have emerged as important biomarkers of resistance for anti-*EGFR* targeted therapies. Further assessment of the molecular mechanisms that dictate this resistance and identification of other specific biomarkers for these agents will provide valuable information to identify the most effective therapy for primary and mCRC patients.

Conference presentations

Shalini Sree Kumar, Jennifer Hardingham. SHC1 and SRC up-regulation contributes to resistance in SW48 treated with anti-*EGFR*. *Poster presentation: Research Day 2011, Basil Hetzel Institute, Adelaide, South Australia, Australia.*

Shalini Sree Kumar, Timothy Price, Jennifer Hardingham. Biomarkers of resistance to anti-*EGFR* therapy in colorectal cancer. *Poster presentation: 24th Lorne Cancer Conference, Lorne, Victoria, Australia.*

Shalini Sree Kumar, Timothy Price, Jennifer Hardingham. *KRAS G13D* mutant colon cancer cell lines - resistant or sensitive to anti-*EGFR* antibody? *Poster presentation: Australasian Gastro-Intestinal Trials Group 14th Annual Scientific Meeting, Sydney, New South Wales, Australia.*

Shalini Sree Kumar, Timothy Price, Jennifer Hardingham. Validation of predictive biomarkers of resistance to anti-*EGFR* in wild type *KRAS*/*BRAF* colorectal cancer cell lines. *Oral presentation: Research Day 2012, Basil Hetzel Institute, Adelaide, South Australia, Australia.*

Shalini Sree Kumar, Timothy Price, Jennifer Hardingham. Validation of predictive biomarkers of resistance to anti-*EGFR* in wild type *KRAS*/*BRAF* colorectal cancer cell lines. *Poster presentation: 6th Australian Health and Medical Research Congress 2012, Adelaide, South Australia, Australia.*

Shalini Sree Kumar, Timothy Price, Jennifer Hardingham. Biomarkers of resistance to anti-*EGFR* in wild type *KRAS*/*BRAF* colorectal cancer cell lines. *Poster presentation: Centre of Personalised Cancer Medicine Symposium 2013, Adelaide, South Australia, Australia.*

Shalini Sree Kumar, Timothy Price, Jennifer Hardingham. Biomarkers of resistance to anti-*EGFR* in wild type *KRAS*/*BRAF* colorectal cancer cell lines. *Poster presentation: Faculty of Health Sciences Postgraduate Research Conference 2013, Adelaide, South Australia, Australia.*

Shalini Sree Kumar, Timothy Price, Omar Mohyieldin, Matthew Borg, Amanda Townsend, Jennifer Hardingham. *KRAS G13D* mutations associated with sensitivity to cetuximab or panitumumab treatment in colorectal cancer cell lines. *Poster presentation: European Cancer Congress 2013, Amsterdam, Netherlands.*

Shalini Sree Kumar, Timothy Price, Jennifer Hardingham. Biomarkers of resistance to anti-*EGFR* in wild type *KRAS*/*BRAF* colorectal cancer cell lines. *Poster presentation: European Cancer Congress 2013, Amsterdam, Netherlands.*

Shalini Sree Kumar, Timothy Price, Jennifer Hardingham. Biomarkers of resistance to anti-*EGFR* in wild type *KRAS*/*BRAF* colorectal cancer cell lines. *Poster presentation: Research Day 2013, Basil Hetzel Institute, Adelaide, South Australia, Australia.*

Prizes awarded

- SCHOOL OF MEDICAL SCIENCES TRAVEL AWARD: Recipient of School of Medical Sciences travel award, AUD$ 2750. Awarded by University of Adelaide, Australia to attend and present at the 2013 European Cancer Congress in Amsterdam and laboratory meetings in University of Amsterdam and Institute of Cancer Research, University of London.

- WINNER FOR BEST POSTER PRESENTATION: Best poster winner in the 2013 Research Day conference, Basil Hetzel Institute for Translational Health Research, Woodville, Australia.