

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

Thesis submitted for the degree of Doctor of Philosophy

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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.

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

~ William Shakespeare

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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.*

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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 Mohyeldin, 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.*

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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

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