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

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Shalini Sree Kumar
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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. 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. 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.*


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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.