Mechanisms of Epigenetic Regulation of Gene Expression in Colorectal Cancer Cells

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Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma 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. 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.

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Date David Mossman

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Table of Contents

Common Abbreviations

ChIP	Chromatin Immunoprecipitation
CpG	CpG dinucleotide sequence
CRC	Colorectal Cancer
DNA	Deoxyribonucleic Acid
DNMT	DNA Methyltransferase
5-aza-dC	5-aza-2-deoxycytidine
HPLC	High Performance Liquid Chromatography
H3	Histone H3
K	Lysine
MDS	Myelodysplastic Syndromes
me	methyl
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
TSA	Trichostatin A

Publications

A. Papers

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4. Mossman D & Scott, RJ, *Long term transcriptional reactivation of epigenetically silenced genes in colorectal cancer cells requires DNA hypomethylation and histone acetylation. Accepted PLoS One,* July 2011.

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B. Conference Proceedings

1. David Mossman & Rodney Scott, 'Prolonged transcriptional reactivation of epigenetically silenced genes requires hypomethylated CpG sites and histone acetylation', Epigenetics 2009, December 1-4, Melbourne, Australia.

2. David Mossman, Cliff J Meldrum & Rodney J Scott, 'Chromatin structure, DNA methylation and its relationship to gene expression'. Poster presentation at 'Stem Cells, Cancer and Aging', September 29-October 4 2008, Singapore, Singapore.

3. David Mossman, Cliff J Meldrum & Rodney J Scott, 'Chromatin structure, DNA methylation and its relationship to gene expression'. Poster presentation at 'Ten of the Best Research Showcase', September 26 2008, University of Newcastle, Callaghan, Australia.

4. David Mossman, Cliff J Meldrum & Rodney J Scott, 'Chromatin structure, DNA methylation and its relationship to gene expression'. Poster presentation at 'Hunter Medical Research Institute Conference on Translational Cancer Research', September 11-12 2008, Newcastle, Australia.

5. David Mossman & Rodney J Scott, ‘Cancer cells differ in their ability to perform DNA methylation which may be responsible for tumour development’. Poster presentation at the ‘Australian Society of Medical Research Meeting’, June 2 2008, Sydney, Australia.

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7. Nicholas Wong, John Heath, Elizabeth Agar, David Mossman, Rodney J Scott, Jeffery Craig, Richard Saffery and David Ashley, ‘Uncovering epigenetic changes in paediatric Acute Lymphoblastic Leukemia’, Gordon Research Conference, Cancer Genetics & Epigenetics, May 20-25, 2007, Il Ciocco, Lucca (Barga), Italy.

8. David Mossman and Rodney J. Scott, ‘Global DNA Methylation levels and response to treatment with a methyltransferase inhibitor’. Oral presentation at the ‘HMRI Cancer Research Program Meeting’, March 15-16, 2007, Newcastle, Australia.

9. David Mossman and Rodney J. Scott. ‘Global methylation analysis by High Performance Liquid Chromatography’. Oral presentation at ‘Graduate Students Day’, October 20, 2006, University of Newcastle, Australia.

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Abstract

The role of epigenetics in disease, particularly cancer, has been an emerging issue for the last decade. For disorders with a genetic component, it offers an alternative mechanism by which disease can initiate and progress. The involvement of epigenetic aberrations in malignancy is evident, with essentially all tumour types displaying variation from a normal epigenetic pattern. A great deal of knowledge can be gained by understanding the epigenetic processes within cells, and manipulation of these mechanisms may lead to more effective treatments and better outcome for individuals at risk of developing cancer.

Studies described in this thesis are aimed to better understand the processes of epigenetic control on gene expression and how they relate to colorectal cancer. Previous studies have identified a single nucleotide polymorphism in *DNMT3B* which is thought to alter the age of disease onset in individuals susceptible to colorectal cancer. The effect of this heritable genetic marker was examined in a larger population size and was found to have no effect on the age of disease onset. This study is described in Chapter 2, the results of which spawned an in-depth analysis of epigenetic change in colorectal cancer cell lines.

The process of DNA methylation was examined, whereby 5-aza-dC was used to demethylate DNA in cultured colorectal cancer cell lines. When the drug was removed from growth medium, inhibition of methyltransferases ceased and remethylation occurred. The resulting effect of gene expression was found to be dependent on initial DNA methylation patterns, and is described in Chapter 3. A

follow up study to this was undertaken to understand the interaction between DNA methylation and histone modifications. The differences between short term and long term reactivated genes after 5-aza-dC exposure depends on increased Histone H3 acetylation and localised hypomethylation. This study is described in Chapter 4.

An investigation of the gene expression profile changes in colorectal cancer cells after 5-aza-dC exposure is described in Chapter 5. A pattern of gene expression similar to healthy epithelial cells was not observed immediately, or ten days after 5-aza-dC treatment. A gene from the Protein Kinase C family was found to be commonly down-regulated with drug treatment. This may have pro-apoptotic effects however this may not be sufficient to induce cell death in these cells as 5-aza-dC is not an effective treatment in solid tumours.

The information described in this thesis will contribute to understanding the process of aberrant DNA methylation that is observed in tumour cells. Information of this nature may identify individuals who are genetically susceptible to the epigenetic inactivation of crucial genes. A complete understanding of the co-ordination of the regulatory proteins will enable more effective treatments against this aspect of malignancy.