

**The Effect of Folic Acid and Methionine Deficiency and Excess  
on DNA Damage and Cancer Growth in HT29 Colon Cancer Cells  
and the Apc Min Mouse Model**

A thesis submitted to the University of Adelaide  
for the degree of Doctor of Philosophy

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## TABLE OF CONTENTS

ABSTRACT	viii
DECLARATION	x
ACKNOWLEDGEMENTS	xii
PRESENTATION AND PUBLICATION ARISING FROM THE THESIS	xiv
LIST OF ABBREVIATIONS	xv
<b>Chapter 1: General Introduction</b>	<b>1</b>
1.1 Colorectal Cancer	1
1.2 Genetic Factor Affecting Colorectal Cancer	6
<b>Chapter 2: Review: The Influence of Folate and Methionine on Intestinal Tumour Development in the Apc<sup>Min/+</sup> Mouse Model</b>	<b>7</b>
2.1 Abstract	10
2.2 Introduction	11
2.2.1 Background	11
2.3 The Apc <sup>Min/+</sup> Mouse Model of Intestinal Cancer	16
2.4 Literature Search and Inclusion Criteria	19
2.5 Studies Meeting the Inclusion Criteria	20
2.5.1 Studies Which Investigated Folate Deficiency Only	26
2.5.2 Studies with Both Folate Deficiency and Excess	30
2.5.3 Study with Folate as a Multivitamin Component	34
2.5.4 Study with Methionine	35

2.6	Proposed Mechanisms for the Effects of Folate and Methionine on Intestinal Tumour Growth in Apc <sup>Min/+</sup> Mice	35
2.6.1	Modulation of Nucleotide Synthesis Caused by Folate Deficiency or Excess	36
2.6.2	DNA Methylation Changes Due to Deficiency of Methyl Donors Which May Alter Gene Expression	38
2.6.3	Altered Polyamine Metabolism Which May Affect the Rate of Cellular Division	39
2.7	Knowledge Gaps and Conclusions	40
<b>Chapter 3: Hypotheses and Objectives</b>		<b>51</b>
3.1	Hypotheses	51
3.2	Aims	52
<b>Chapter 4: <i>In Vitro</i> Studies – I</b>		
<b>The Effect of Folic Acid and Methionine on Cell Growth and Genome Stability in HT29 Cells</b>		<b>53</b>
4.1	Objective	53
4.2	Hypotheses	53
4.3	Introduction	53
4.4	Methods and Preliminary Experiments	55
4.4.1	HT29 Cell Culture	55
4.4.2	Preparation of Medium with Different Folic Acid and Methionine Concentrations	56

4.4.3	HT29 Cell Proliferation	58
4.4.3.1	Growth Observation of 14 Day Culture of HT29 in 96 Well Plates	58
4.4.3.2	HT29 Cell Proliferation in Various Folic Acid and Methionine Concentrations	60
4.4.4	Genomic Stability and Cytostasis of HT29 Cells	62
4.4.4.1	Preliminary Analysis of Growth Observation of 14 Day Cultures of HT29 in 24 Well Plates	62
4.4.4.2	DNA Stability in HT29 in Various Folic Acid and Methionine Concentrations	65
4.4.5	CBMN-Cyt Assay	65
4.4.6	Statistical Analyses	73
4.5	Results	74
4.5.1	HT29 Cell Proliferation	74
4.5.2	DNA Stability in HT29 Cells	83
4.5.2.1	Nuclear Division Index (NDI)	83
4.5.2.2	Apoptosis	86
4.5.2.3	Necrosis	86
4.5.2.4	Binucleated Cells with Micronuclei	90
4.5.2.5	Binucleated Cells with Nucleoplasmic Bridges	90
4.5.2.6	Binucleated Cells with Nuclear Buds	91
4.6	Discussion	95

**Chapter 5: *In Vitro* Studies – II**

	<b>The Effect of Folic Acid and Methionine on Telomere Length and DNA Methylation in HT29</b>	<b>99</b>
5.1	Objective	99
5.2	Hypotheses	99
5.3	Introduction	99
5.4	Material and Methods	102
5.4.1	Study Design	102
5.4.2	14 Day Culture of HT29 Cells	104
5.4.3	DNA Isolation	104
5.4.4	Telomere Length Assay	106
5.4.4.1	Quantitative Real-time Polymerase Chain Reaction (qPCR) for Telomere Length	106
5.4.4.2	qPCR for 36B4 Single Copy Gene (SCG)	107
5.4.4.3	Calculation of Telomere Length	107
5.4.5	DNA Methylation Assay	108
5.4.6	Statistical Analyses	109
5.5	Results	110
5.5.1	Telomere Length	110
5.5.2	DNA Methylation	115
5.5.3	Correlation	120
5.6	Discussion	120

**Chapter 6: *In Vivo* Studies – I**

**The Effect of Folate and Methionine on Cancer Development in  
the Apc<sup>Min/+</sup> Mouse Model**

		<b>125</b>
6.1	Objective	125
6.2	Hypotheses	125
6.3	Introduction	125
6.4	Material and Methods	127
6.4.1	Mouse Model	127
6.4.2	Genotyping	128
6.4.3	Study Design	129
6.4.4	Diet	133
6.4.5	Animal Welfare	136
6.4.6	Body Weight	138
6.4.7	Food Intake Observation	138
6.4.8	Sacrifice of Mice at End of Experiments	138
6.4.9	Organs Weight and Size	139
6.4.10	Haematocrit Measurement	139
6.4.11	Plasma Methionine and Folate Measurement	139
6.4.12	Tumour Measurement in APC <sup>Min/+</sup> Mice	140
6.4.13	Tissue Folate and Methionine Measurement	141
6.4.14	Statistical Analyses	143
6.5	Results	144
6.5.1	Body Weight	144
6.5.2	Food Intake	144

6.5.3	Organ Weight and Size	147
6.5.4	Haematocrit Level	150
6.5.5	Plasma Folate and Methionine	153
6.5.6	Folate and Methionine Concentration in Mucosal Tissue of the Small Intestine (MTSI)	156
6.5.7	Intestinal Tumour Incidence in the $Apc^{Min/+}$ mice	161
6.6	Discussion	167
6.6.1	Strength of study	172
6.6.2	Weakness of study	172
6.6.3	Conclusion	173
<b>Chapter 7: <i>In Vivo</i> Studies – II</b>		
<b>The Effect of Folic Acid and Methionine on Genomic Instability in the <math>Apc^{Min/+}</math> Mouse Model</b>		
		<b>174</b>
7.1	Objective	174
7.2	Hypotheses	174
7.3	Introduction	174
7.4	Material and Methods	176
7.4.1	Mouse Model and Dietary Regimen	176
7.4.2	Study Design	177
7.4.3	Blood Collection for Whole Blood Micronucleus Erythrocyte Assay	179
7.4.3.1	Acridine Orange Staining	179
7.4.4	DNA Isolation from the Colon Tissue	182
7.4.5	Telomere Length Assay	183

7.4.5.1	Quantitative Real-time Polymerase Chain Reaction (qPCR) for Telomere Length	183
7.4.5.2	qPCR for 36B4 Single Copy Gene (SCG)	183
7.4.5.3	Calculation of Telomere Length	183
7.4.6	DNA Methylation Assay	184
7.4.7	Statistical Analyses	184
7.5	Results	185
7.5.1	Whole Blood Micronucleus Erythrocyte Assay	185
7.5.1.1	Ratio of Polychromatic Erythrocytes (PCE) to Non Polychromatic Erythrocytes (NCE)	185
7.5.1.2	Micronucleated Non Polychromatic Erythrocytes (MN-NCE)	186
7.5.1.3	Micronucleated Polychromatic Erythrocytes (MN-PCE)	187
7.5.2	Telomere Length	192
7.5.3	DNA Methylation	196
7.5.4	Correlation	200
7.6	Discussion	202
<b>Chapter 8:</b>	<b>Conclusion, General Discussion &amp; Future Direction</b>	<b>207</b>
	References	213
	APPENDIX: PAPER REPRINTS	227



## ABSTRACT

Folate and methionine are critical for one-carbon metabolism, impacting DNA synthesis, repair, and methylation processes, as well as polyamine synthesis. These micronutrients have been implicated in colorectal cancer risk. The aim of this thesis was to examine in greater detail the role of folate and methionine in colon cancer initiation and progression by assessing DNA stability and tumour incidence. Studies were performed *in vitro* (using human colorectal adenocarcinoma HT29 cell line) and *in vivo* (using *Apc*<sup>Min/+</sup> mouse model).

The *in vitro* studies examining the effects of various folic acid and methionine concentrations within the physiological range on cell proliferation and genomic instability of HT29 cells, showed that restriction of folic acid or methionine inhibited cellular proliferation, while supra-physiological folate induced apoptosis. HT29 cells may be resistant to genome instability induced by folic acid or methionine deficiency under the experimental conditions reported for this study because no significant increases in micronuclei, nuclear buds or nucleoplasmic bridges were observed in the Cytokinesis-block micronucleus cytome (CBMN-Cyt) assay. The investigation on the effect of folic acid and methionine depletion on telomere length and DNA methylation in HT29 cells demonstrated that folate and methionine depletion may increase both telomere length and DNA methylation in HT29 cells. The length of telomere was positively correlated with DNA methylation.

In the *in vivo* studies using the *Apc*<sup>Min/+</sup> mouse model, the effect of supplementing a western-style diet with dietary folic acid and/or methionine on intestinal tumour development was assessed. A total of 113 mice were randomised to receive one of the four diet treatments; New Western Diet (NWD) as control diet, NWD with additional folic acid, NWD with additional methionine, and NWD with additional folic acid and methionine, administered at age of 3 until 13 weeks, with wild type (WT) mice used as controls. Supplementation of folic acid and methionine separately, resulted in marginally lower tumour numbers, when compared to the control diet. However, supplementation with both folic acid and methionine together appeared to annul the

marginal protective effect of supplementing individually. The investigation on the effect of supplementing a western-style diet with dietary folic acid and/or methionine on genomic stability (measured via micronucleated erythrocyte assay on blood sample; telomere length and DNA methylation on the colon tissue) showed insufficient evidence that additional folic acid and/or methionine promotes DNA stability or instability in  $Apc^{Min/+}$  or WT mice. Dietary supplementation with folic acid and/or methionine at the levels and duration used in this study did not substantially promote or protect against DNA damage in WT or intestinal cancer-prone  $Apc^{Min/+}$  mouse model fed a western-style diet although a marginal effect on tumour number was evident.

In conclusion, the results of this thesis support a role of methionine and folate in affecting intestinal cell proliferation and possibly tumour number. However, the impact of supplementation with folate and methionine on genome stability was marginal.

## DECLARATION

I certify that this work 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.

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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ARNIDA HANI TEH

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## PRESENTATION AND PUBLICATION ARISING FROM THE THESIS

### Abstract/Poster Presentation

1. **Arnida Hani Teh**, Erin Symonds, Peter Clifton and Michael Fenech. Effects of Low Folate on Cell Growth and Genome Stability in HT29 Cell Lines. 19th International Conference on Nutrition. 4-9th October 2009, Bangkok, Thailand.

### Publication

1. **Teh, A.H.**, Symonds, E., Clifton, P. & Fenech, M. *The influence of folate and methionine on intestinal tumour development in the  $Apc^{Min/+}$  mouse model.* Mutation Research/Reviews in Mutation Research, 2012. **751**(1): p. 64-75.

## LIST OF ABBREVIATIONS

5,10-MeTHF	5,10- methylenetetrahydrofolate
5-MeTHF	5-methyltetrahydrofolate
ACF	aberrant crypt foci
AHT	Arnida Hani Teh
ALT	alternative lengthening of telomeres
ANOVA	analysis of variance
AOM	azoxymethane
Apc	adenomatous polyposis coli
BER	base excision repair
BHMT	betaine:homocysteine methyltransferase
BN	binucleated
CB	Caroline Bull
CBMN Cyt assay	Cytokinesis Block Micronucleus Cytome assay
Cq	cycle threshold
CSIRO	Commonwealth Scientific and Industrial Research Organisation
Cyto-B	cytochalasin-B
dcSAM	decarboxylated SAM
DFMO	$\alpha$ -difluoromethylornithine
DHF	dihydrofolate
DMG	dimethylglycine
DNA	deoxyribonucleic acid
DNMT1	DNA (cytosine-5-)-methyltransferase 1



DSH	dishevelled
dTMP	deoxythymidine monophosphate
dTTP	deoxythymidine triphosphate
dUMP	deoxyuridine monophosphate
FAD	flavin adenine dinucleotide
FAP	familial adenomatous polyposis
FDA	Food and Drug Administration
FDR	false discovery rate
Folbp1	folate binding protein
GSK 3	glycogen synthase kinase 3
LRP	LDL receptor related protein
MF	Michael Fenech
MMRs	mismatch repair enzymes
MNi	micronuclei
MN-NCE	micronucleated normochromatic or non polychromatic erythrocytes
MN-PCE	micronucleated polychromatic erythrocytes
MTAP	methylthioadenosine phosphorylase
Mthdf1	methylenetetrahydrofolate dehydrogenase
MTHFR	methylenetetrahydrofolate reductase
MTR	methionine synthase
MTSI	mucosal tissue of the small intestine
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NBUDs	nuclear buds

NCE	normochromatic or non polychromatic erythrocytes
NDI	nuclear division index
NPBs	nucleoplasmic bridges
NWD	New Western Diet
NWD+FA	New Western Diet with additional folic acid
NWD+FA+M	New Western Diet with additional folate and methionine
NWD+M	New Western Diet with additional methionine
OD	optical density
PBS	phosphate-buffered saline
PCE	polychromatic erythrocytes
qPCR	Quantitative Real-time Polymerase Chain Reaction
Rfc1	reduced folate carrier 1
RNA	ribonucleic acid
RPMI	Roswell Park Memorial Institute
SAH	S-adenosyl homocysteine
SAM	S-adenosyl methionine
SAMDC	S-adenosyl methionine decarboxylase
SCG	single copy gene
SD	standard deviation
SE	standard error
SHMT1	cytoplasmic serine hydroxymethyltransferase
SHMT1	serine hydroxymethyltransferase
TCF	T-cell transcription factor
THF	tetrahydrofolate

TS	thymidylate synthase
USA	United States of America
WT	wild type