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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November 2016

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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 $Apc^{Min/+}$ 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 $Apc^{Min/+}$ 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.

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ACKNOWLEDGEMENTS

Praise to God the Almighty for the successful completion of this thesis. I am grateful to be given the strength to make this materialise eventually.

My sincere appreciation to my highly respectable principle supervisor; Professor Michael Fenech and astonishing co-supervisors, Dr. Caroline Bull and Professor Peter Clifton for the guidance and opportunities given. The knowledge and experience gained are truly appreciated.

I would like to thank these nice people who helped me in this study; Carolyn Salisbury, Theodora Almond and Maryam Hor for helping me in *in vitro* studies and CBMN-Cyt assay; Dr. Erin Symonds, Darien Sander, Ben Scherer, Dr. Varinderpal Dhillon, Dr. Phil Thomas, Dr. Damien Belobrajdic and Thelma Bridle for helping me with the *in vivo* studies; Dr. Nathan O'Callaghan for helping me in telomere length assay; and Kylie Lange for helping me with statistical analysis.

Special thanks to my parents that never tired of giving support and pray for my success. I am also grateful for the friends that crosses paths; Razinah, Sau Lai, Mansi, Jing Wu, Carly, Anne, Penny, Sarah, Sabbir, Awan, Zati, Min, Yanti, Rafisah, Aida, Fauziah, Liyana, Nadiah, Nadia and Izzah. Thank you for the friendship and company that I never felt alone in this PhD journey.

I would also like to acknowledge CSIRO for the studentship opportunity, and to my employer (the National University of Malaysia) and the government of Malaysia (Ministry of Higher Education) for the scholarship provided.

The journey was long and winding. I fell and able to get up again with all help and support that I received. Thank you so much.

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	α-difl uoromethylornithine
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