Do dietary induced pro-mutagenic DNA adducts increase risk for colorectal cancer?

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Summary

Colorectal cancer (CRC) is a major burden on public health in developed countries with high incidence and mortality rates globally. A major driving force of CRC is related to lifestyle factors, in particular dietary choices. Consumption of red meat has been identified as a risk factor for developing CRC by the World Cancer Research Fund. Increased DNA adducts in the colon via excess endogenous N-nitrosation is one mechanism thought to play a role in colorectal oncogenesis. Haem iron in red meat has also been implicated in development of CRC in humans. A dietary component that can protect against CRC, called resistant starch (RS), is the component of starch undigested in the small intestine and fermented in the colon. It is thought that preferential fermentation of carbohydrate over protein, when RS is incorporated into high protein diets, leads to a reduction in DNA changes that might initiate CRC. Green tea is a common beverage in East Asian countries and evidence from rodent and cell culture studies shows green tea as a preventative agent against CRC, although human studies are somewhat conflicting.

The global aim of this thesis is to determine whether dietary-induced DNA adducts by red meat consumption act as bio-markers for risk of CRC. The studies presented will endeavour to validate and extend previous studies demonstrating that red meat can induce pro-mutagenic adducts. Furthermore, RS and green tea will be employed in combination with red meat to ascertain any protective role they might have against pro-mutagenic formation in the colon. The risk of developing CRC with high red meat consumption will also be explored, and RS will be evaluated as a protective food against CRC formation. The hypotheses are that red meat will increase DNA adducts, but that RS and green tea consumption can reduce red meat-induced DNA adducts. Also, red meat and haem from red meat will increase risk for developing CRC, but RS will reduce the CRC risk posed by red meat. In addition to the mouse experiments, the effects of red meat and red meat in combination with RS will be translated to the human setting, by feeding high red meat and high red

meat with RS diets to healthy human volunteers. It is hypothesised that red meat will significantly increase DNA adducts in the colorectal tissue of humans consuming a high red meat diet, but that co-consumption with RS will ameliorate these adducts.

Red meat and haem increased DNA adducts of the distal colon in all mouse models and in human rectal epithelial tissue. However, there was no clear link between DNA adducts and risk for oncogenesis of the colon in the mouse models tested. RS increased fermentation of beneficial microbial metabolites, but reduced production of potentially toxic fermentation products. RS reduced proliferation rates in the distal colon of wild type and *Msh2* knockout mice, but this did not reduce pre-cancerous lesions in the colon. RS supplementation could reduce formation of pro-mutagenic adducts in wild type mice and in humans after short term consumption, but this did not translate over long term RS consumption in the Western diet mouse model. Green tea did not reduce DNA adducts either alone or in the presence of red meat, either in wild type or *MGMT* knockout mice.

In conclusion, chronic consumption of a high red meat diet can generate DNA lesions in colonic epithelial cells and RS consumption can ameliorate this affect in the short term, but this does not lead to consequent changes in risk after long term consumption in the mouse models tested. Consequently, dietary-induced DNA O^6MeG and 8-oxo adducts could perhaps be described as a marker for exposure to alkylating and oxidative agents in the diet, including red meat and its associated components such as haem, and not necessarily described as a bio-marker for CRC risk.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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Abbreviations

8-oxo	8-hydroxy-2'-deoxyguanosine
AAR	Acute apoptotic response
ACF	Aberrant crypt foci
AIHW	Australian Institute of Health and Welfare
AIN	American Institute of Nutrition
AOM	Azoxymethane
ATase	Alkyl-guanine-alkyl-transferase
BER	Base excision repair
BG	O^6benzylguanine
BMI	Body mass index
BCFA	Branched chain fatty acids
CD	Chrohn's disease
CIMP	CpG island methylator phenotype
CIN	Chromosomal instability
CRC	Colorectal cancer
CSIRO	Commonwealth Scientific and Industrial Organisation
DAB	3'-diaminobenzamine
DMH	1,2-dimethylhydrazine
DSS	Dextran sodium sulphate
EGCG	(-)-epicatechin-3-gallate
FAP	Familial adenomatous polyposis
FCC-X	Familial colorectal cancer type X
FIT	Faecal immunochemical test
H_2O_2	Hydrogen peroxide
H&E	Haematoxylin and Eosin
HAMSB	Butyrylated high amylose maize starch
HCA	Heterocyclic amine
HNPCC	Hereditary non-polyposis colorectal cancer
HR	Homologous recombination
HRM	High red meat
HRP	Horse radish peroxidase
IACR	International Agency on Cancer Research

IBD	Inflammatory bowel disease
IHC	Immunohistochemical
IQ	2-amino-3methylimidazo [4,5-f] quinoline
IS	Internal standard
LOH	Loss of heterozygosity
MSS	Microsatellite stable
MSI	Microsatellite unstable
MIN	Microsatellite instability
MMR	Mismatch repair
MAM	Methylazoxymethanol
MeIQ	2-amino-3,8-dimethylimidazo[4,5-*f*]quinoline
MGMT	Methyl-guanine-methyl-transferase
MNU	Methylnitrosourea
MoM	Mouse-on-mouse
mutSα	*Msh2-Msh6* MMR heterodimer complex
NHEJ	Non-homologous end joining
NOCs	N-nitroso compounds
O_2^-	Superoxide anion
O^6CMG	O^6-Carboxymethlyguanosine
O^6meG	O^6methyl-2-deoxyguanasine
PAH	Polyaromatic hydrocarbon
PBS	Phosphate buffered saline
PCNA	Proliferating cell nuclear antigen
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
RoB	Red over blue
ROS	Reactive oxygen species
RS	Resistant starch
RT	Room temperature
SCFA	Short chain fatty acid
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick-end labelling
UC	Ulcerative colitis
WCRF	World Cancer Research Fund

Published material and presentations arising from this thesis

Peer reviewed publications

1. *Karen J Humphreys, Michael A Conlon, Graeme P Young, David L Topping, Ying Hu, **Jean Winter**, Anthony R Bird, Lynne Cobiac, Nicholas A Kennedy, Michael Z Michael and Richard K Le Leu. (2014) Dietary manipulation of oncogenic microRNA expression in human rectal mucosa: a randomised trial. *Can. Prev. Res.* 7(8); 786–95
2. **Jean Winter**, Graeme P Young, Ying Hu, Silvia W Gratz, Michael A Conlon and Richard K Le Leu (2013) Accumulation of promutagenic DNA adducts in the mouse distal colon after consumption of heme does not induce colonic neoplasms in the western diet model of spontaneous colorectal cancer. *Mol. Nut. Food. Res.* 58(3):550-8.
3. **Jean M Winter**, Ying Hu, Graeme P Young, Maija RJ Kohonen-Corish, Richard K Le Leu, Role of red meat and resistant starch in pro-mutagenic adduct formation, thymic lymphoma and intestinal tumourigenesis in *Msh2* deficient mice. *Jrnl. Nutrigenetics and Nutrigenomics,* In press 2015

Abstract publications

1. **Jean M. Winter**, Ying Hu, Graeme P Young, Maija RJ Kohonen-Corish, Richard K Le Leu. Diverse effects of resistant starch and red meat on proliferation and O^6Methyl-2-deoxyguanasine adduct formation in the distal colon of Msh2 deficient mice: Consequences for colorectal carcinogenesis. 38th Congress of the International Society of Nutrigenetics/Nutrigenomics (ISNN). May 2-3, 2014 Gold Coast, Australia: Abstracts. J Nutrigenetics Nutrigenomics 2014;7:1-38 (DOI:10.1159/000362615) (Abstract Only)
2. Richard K Le Leu, **Jean M Winter**, Ying Hu, Laura S Nyskohus, Michael Conlon, Anthony R Bird, David L Topping, Graeme P Young. M1181 Red Meat Diets Increase the Formation of O^6Methyl2Deoxyguanosine Adducts in the Mouse Colon: Attenuation by Resistant Starch. Gastroenterology. 2010; 138(5). DOI:10.1016/S0016-5085(10)61607-1 (Abstract Only)

Publications currently under peer review

1. Richard K Le Leu, **Jean M Winter**, Karen J Humphreys, Graeme P Young, Claus T Christophersen, Ying Hu, Silvia W Gratz, Rosalind B Miller, David L Topping, Anthony R Bird, Michael A Conlon, Butyrylated starch intake can prevent red meat induced O^6-methyl-2-deoxyguanosine adducts in human rectal tissue: a randomised clinical trial. *British J. Nut.*

*J. Winter performed DNA adduct and proliferation laboratory analyses of rectal epithelial tissue samples for this project

National conference presentations

1. Poster Presentation: **Australian Health and Medical Research Congress**, Melbourne, November 2014, Methyl-guanine-methy-transferase repairs pro-mutagenic adducts and influences the acute apoptotic response to alkylating agents: Interactions of dietary red meat, green tea and gender.
2. Oral Presentation: **Australian Society for Medical Research SA Annual Scientific Meeting**, Adelaide, June 2014, Diverse effects of resistant starch and red meat on proliferation and O^6Methyl-2-deoxyguanasine adduct formation in the distal colon of Msh2 deficient mice: Consequences for colorectal carcinogenesis.
3. Oral Presentation: **Australian Society for Medical Research SA Annual Scientific Meeting**, Adelaide, June 2013, Accumulation of pro-mutagenic and oxidative DNA adducts in the distal colon after consumption of dietary haem does not increase colorectal cancer in the mouse.
4. Oral Presentation: **Australian Society for Medical Research SA Annual Scientific Meeting**, Adelaide, June 2012, Induction of Pro-mutagenic Adducts in the Colon and Risk for Colorectal Cancer: Regulation by Resistant Starch.

International conference presentations

1. Oral Presentation (Registration waived): **8th Congress of International Society of Nutrigenetics/Nutrigenomics (ISNN),** Gold Coast, QLD, Australia, May 2014 Diverse effects of resistant starch and red meat on proliferation and O^6Methyl-2-deoxyguanasine adduct formation in the distal colon of Msh2 deficient mice: Consequences for colorectal carcinogenesis.
2. Oral and Poster Presentation: **Environmental Mutagen Society Annual Meeting**, Seattle, Washington, USA, September 2012, High Dietary Protein and DNA Damage in the Mouse Colon and Human Rectal Epithelium: Regulation by Resistant Starch**.**