Dietary regulation of microRNA expression in colorectal cells

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List of Abbreviations

Ac	acetylation
ACTB	beta actin
ACVR2	activin type 2 receptors
AGO2	argonaute-2
AKT (PKB)	v-akt murine thymoma viral oncogene homolog 1
ANOVA	analysis of variance
APC	adenomatous polyposis coli
APS	ammonium persulfate
Arg	arginine
BAX	BCL2-associated X protein
BCL2L11 (Bim)	BCL2-like 11 (apoptosis facilitator)
BR	butyrate-resistant
BRAF	v-Raf murine sarcoma viral oncogene homolog B1
C13ORF25	C13 open reading frame 25
CAS	cellular apoptosis susceptibility
CCDC88A	coiled-coil domain containing 88A
CCND1	cyclin D1
CCNE1	cyclin E1
CDK19 (CDC2L6)	cyclin-dependent kinase 19
CDK8	cyclin-dependent kinase 8
CDKN1A (p21)	cyclin-dependent kinase inhibitor 1A
CDKN2A	cyclin-dependent kinase inhibitor 2A
cDNA	complementary DNA
CDX2	caudal type homeobox 2
ChIP	chromatin immunoprecipitation
CHX	cycloheximide
CIMP	CpG island methylator phenotype
CIN	chromosomal instability
CRC	colorectal cancer
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CTGF	connective tissue growth factor
CTNNB1	catenin (cadherin-associated protein), beta 1
DALY	disability-adjusted life year

DGCR8	DiGeorge syndrome critical region gene 8
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNMT1	DNA (cytosine-5-)-methyltransferase 1
DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha
DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta
dsRNA	double stranded RNA
DTT	dithiothreitol
E2F	E2F transcription factor family
E2F1	E2F transcription factor 1
E2F2	E2F transcription factor 2
E2F3	E2F transcription factor 3
ECL	enhanced chemiluminescence
EDTA	ethylenediaminetetraacetic acid
EGR2	early growth response 2
EMAST	elevated microsatellite instability at selected tetranucleotide repeats
EPIC	European Prospective Investigation into Cancer and Nutrition
ESR1	estrogen receptor 1
FAP	familial adenomatous polyposis
FBXW7	F-box and WD repeat domain containing 7, E3 ubiquitin protein ligase
FDA	US Food and Drug Administration
FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog
FSANZ	Food Standards Australia New Zealand
GAB1	GRB2-associated binding protein 1
GAB2	GRB2-associated binding protein 2
GADD45A	growth arrest and DNA-damage-inducible, alpha
H1/H5	histone 1/ histone 5
H2A and 2B	histone 2A and 2B
H3	histone 3
H4	histone 4
HAT	histone acetyltransferase
HER2 (ERBB2)	human epidermal growth factor receptor 2
HDAC	histone deacetylase
HDI	histone deacetylase inhibitor

HDM	histone demethylases
His	histidine
HMT	histone methyltransferase
HNPCC	hereditary nonpolyposis colorectal cancer
hnRNPA1	heterogeneous nuclear ribonucleoprotein A1
HRAS	v-Ha-ras Harvey rat sarcoma viral oncogene homolog
IgG	immunoglobulin G
IP	immunoprecipitation
IPA	Ingenuity Pathway Analysis
JARID1B	histone demethylase jumonji, AT rich interactive domain 1B
K	lysine
KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
LIN28A	lin-28 homolog A
Lys	lysine
MAP	mitogen-activated protein
MAPK7 (ERK5)	mitogen-activated protein kinase 7
MAPK12	mitogen-activated protein kinase 12
MCC	mutated in colorectal cancers
me	methylation
me2	di-methylation
me3	tri-methylation
MGMT	O6-methylguanine-DNA methyltransferase
MIR17HG	miR-17-92 cluster host gene
miRNA	microRNA
MLH1	DNA mismatch repair protein MLH1 (MutL protein homolog 1)
MLH2	DNA mismatch repair protein MLH2 (MutL protein homolog 2)
MSH2	mutS homolog 2
MSH3	mutS homolog 3
MSH6	mutS homolog 6
MSI	microsatellite instability
MSS	microsatellite stable
MYB	v-myb myeloblastosis viral oncogene homolog
MYC (C-MYC)	myelocytomatosis oncogene
NC	negative control
NEDD9 (HEF1)	neural precursor cell expressed, developmentally down-regulated 9

NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NHMRC	National Health and Medical Research Council
NR	not reported
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
NRM	normal rectal mucosa
NSP	non-starch polysaccharide
O6CMG	O6-carboxymethylguanine
O6MeG	O6-methyl-2-deoxyguanosine
PACT	protein activator of PKR
PBS	phosphate buffered saline
PcG	polycomb group
PDCD4	programmed cell death 4
RHOB	ras homolog family member B
PI3K	phosphatidylinositol 3-kinases
PIC	protease inhibitor cocktail
PIK3CA	phosphoinositide-3-kinase, catalytic, alpha polypeptide
PMS2	postmeiotic segregation increased 2
PMSF	phenylmethylsulfonyl fluoride
pre-miRNA	precursor miRNA
pri-miRNA	primary miRNA
PTEN	phosphatase and tensin homolog
RISC	RNA-induced silencing complex
RM	red meat
RNA	ribonucleic acid
RNAi	RNA interference
RNU6B	U6B small nuclear RNA
RPL30	ribosomal protein L30
RS	resistant starch
RTCA	real-time cell analysis
RT-PCR	reverse transcription polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SAHA	suberoylanilide hydroxamic acid
SCFA	short-chain fatty acid
SD	standard deviation
SEM	standard error of the mean
shRNA	short hairpin RNA

siRNA	small interfering RNA
SIS (PDGFB)	platelet-derived growth factor beta polypeptide
SMAD2	SMAD family member 2
SMAD3	SMAD family member 3
SMAD4	SMAD family member 4
SP1	Sp1 transcription factor
SP3	Sp3 transcription factor
TBS	tris-buffered saline
TCF4	transcription factor 4
TEMED	tetramethylethylenediamine
TGFBIIR	transforming growth factor beta, receptor type II
TNM	Tumour Node Metastasis (TNM) Classification of Malignant Tumours
TP	target protector
TP53	tumour protein p53
TPM1	tropomyosin 1
TRBP	human immunodeficiency virus transactivating response RNA-binding protein
TRxG	trithorax group
TS	thymidylate synthase
TSA	trichostatin A
TSP1	thrombospondin-1
TSS	transcription start site
TXNIP	thioredoxin-interacting protein
UTR	untranslated region
WCRF	World Cancer Research Fund
WNT	wnt signalling protein
XPO5	exportin 5
YLL	years of life lost

Summary

Colorectal cancer (CRC) development is associated with epigenetic modifications, including DNA methylation changes, altered histone modification patterns, and dysregulated microRNA (miRNA) expression. While some dietary compounds can alter colorectal cell behaviour through epigenetic mechanisms, their role in modifying miRNA expression in CRC cells and normal colorectal tissue has been less studied. The diet-derived compound butyrate, with its known role in histone modification, is a plausible candidate for altering miRNA expression. This study examined dietary regulation of miRNA expression in colorectal cells, and explored the role of butyrate and other histone deacetylase inhibitors (HDIs) in modulating CRC risk through altered miRNA expression. The down-stream consequences of these miRNA changes, and the roles of miRNAs in the context of the anti-proliferative effects of HDIs, were determined. In addition to exploring the action of butyrate, a potentially protective dietary component, the study also investigated whether factors that possibly increase CRC risk, such as high red meat intake, alter miRNA expression.

In vitro, butyrate and other HDIs altered levels of some miRNAs that are dysregulated in CRC, including the oncogenic miR-17-92 miRNA cluster which is over-expressed in CRC. Butyrate decreased miR-17-92 miRNA levels in CRC cells, with a corresponding increase in expression of miR-17-92 targets, including cell cycle inhibitors and pro-apoptotic genes. Mechanisms for this decrease included changes in regulators of miR-17-92 host gene transcription, and altered histone acetylation and methylation patterns centred around the transcription start site and promoter of the miR-17-92 host gene. Decreased miR-17-92 expression may be partly responsible for the anti-proliferative effects of HDIs, with introduction of miR-17-92 cluster miRNA mimics reversing this effect and decreasing target gene transcript levels. Of the cluster members, miR-19a and miR-19b were primarily responsible for promoting proliferation, while in a novel finding, miR-18a acted in opposition to other members to decrease growth. Two pro-proliferative genes, *NEDD9* and *CDK19*, were identified as novel miR-18a targets. This study presents the first evidence of competing roles for miR-17-92 cluster members, in the context of HDI-induced changes in CRC. miR-18a may play a homeostatic role in containing the oncogenic effects of the entire cluster, but may be selectively decreased in CRC compared with other cluster members.

SUMMARY

In addition to the capacity of butyrate to reverse the dysregulation of miR-17-92 miRNAs in CRC cells *in vitro*, this action was demonstrated with resistant starch supplementation *in vivo*, in rectal biopsies from healthy human volunteers exposed to high red meat levels. High red meat intake raised levels of miRNAs with oncogenic potential, particularly miR-17-92 cluster miRNAs and miR-21. Resistant starch supplementation raised faecal butyrate concentrations, and decreased miR-17-92 cluster miRNAs to baseline levels. *In vivo* modulation of miRNAs in colorectal cells by dietary compounds has not previously been demonstrated in humans. Regulation of miRNA expression demonstrates a plausible mechanism to explain some of the chemo-protective effects of butyrate, and potentially carcinogenic properties of other dietary components. Understanding how dietary compounds alter miRNA expression, and how miRNAs modulate the action of HDIs, may provide new opportunities for CRC therapies and prevention strategies.

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.

Karen J Humphreys

Publications and Presentations

Peer-reviewed Publications

Humphreys KJ, Cobiac L, Le Leu RK, Van der Hoek MB, Michael MZ. Histone deacetylase inhibition in colorectal cancer cells reveals competing roles for members of the oncogenic miR-17-92 cluster. *Molecular Carcinogenesis*. 5 February 2012. [Epub ahead of print].

Poster Presentations

Humphreys KJ, Le Leu RK, Cobiac L, Michael MZ. Butyrate alters microRNA expression in colorectal cells. 31st Lorne Genome Conference, Mantra Erskine Resort, Lorne, Victoria, Australia, 14 – 16 February 2010.

Humphreys KJ, Le Leu RK, Cobiac L, Michael MZ. Butyrate alters microRNA expression in colorectal cells. MicroRNAs and Non-Coding RNAs and Cancer Keystone Symposia Conference, Fairmont Banff Springs, Banff, Alberta, Canada, 11 – 16 February 2011.

Humphreys KJ, Cobiac L, Le Leu RK, Van der Hoek MB, Michael MZ. Histone deacetylase inhibition in colorectal cancer cells reveals competing roles for members of the oncogenic miR-17-92 cluster. Epigenetics 2012 4th Australian Scientific Conference, the National Wine Centre of Australia, Adelaide, South Australia, Australia, 7 – 9 May 2012. **Best conference poster prize.**

Oral presentations

Humphreys KJ, Le Leu RK, Cobiac L, Michael MZ. Butyrate alters microRNA expression in colorectal cells. Australian Society for Medical Research Annual Scientific Meeting, the Entertainment Centre, Adelaide, South Australia, Australia, 9 June 2010.

Various presentations at the Flinders Centre for Innovation in Cancer annual research days, Adelaide RNA special interest group meetings, and the Flinders Clinical and Molecular Medicine cluster seminar series.

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