Studies into the relationship between GPCR43 and BuA-induced effects on colorectal cancer

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Thesis summary

Colorectal cancer (CRC) is a major problem in affluent countries worldwide. In Australia it is the second most commonly diagnosed malignancy with approximately 13,000 new cases diagnosed each year. This disease is also the leading cause of cancer related death in Australia with approximately 4,500 fatalities each year. Epidemiological studies have shown geographical variation in the incidence of disease, with diet considered to be a key contributing factor to CRC risk. In particular, diets high in fibre and low in fat have been demonstrated to reduce the risk of developing CRC.

Fibre is heterogeneous in nature and can be categorised into different subtypes. Resistant starch is a component of fibre which remains largely intact throughout the gastrointestinal tract until it reaches the colon. Here it undergoes bacterial fermentation to produce the short chain fatty acids (SCFAs) acetate, propionate and butyrate (BuA). Each of the SCFAs are bioactive in the colon, with the most active being BuA. The beneficial effects of fibre have been linked to BuA's ability to induce colon cancer cell differentiation, reduce proliferation and initiate apoptosis. Interestingly, in normal cells BuA is utilised as the preferential energy source and has been shown to promote proliferation. With an apparent "paradoxical effect" on normal and cancerous cells BuA has been the subject of much investigation as a potential anticancer agent. Despite numerous studies investigating BuA actions, the exact biological mechanisms remain largely undefined. This thesis explored a possible mechanism for BuA-induced apoptosis and inhibition of proliferation.

In 2003, two publications provided evidence that SCFAs, including BuA, were ligands to two members of a previously orphan family of G-protein coupled receptors (GPCRs); GPCR41 and 43. Of the two receptors BuA had the strongest effect on GPCR43. Consequently this thesis investigated the possibility that BuA acts to decrease CRC proliferation and induce apoptosis by binding to and activating GPCR43 on CRC cells. It was hypothesised that GPCR43 acted as a "BuA sensor" on the surface of the cell to mediate the effects of BuA. This experimental work utilised PCR, Q-PCR, measures of apoptosis, proliferation and differentiation and RNAi knockdown. The key areas of investigation included:

(1) Determining if GPCR43 was present on a range of CRC cell lines with a cell line to represent adenocarcinoma, carcinoma and metastatic stage of disease.

(2) Investigating the expression of GPCR43 with manipulated nutrient media and different levels of cell confluence.
(3) Exploring GPCR43 expression in normal and malignant human patient biopsies.
(4) Determining if the inhibition of G-protein function using inhibitors influenced BuA-induced changes to apoptosis and proliferation.
(5) Using RNAi, investigating the effect that GPCR43 knockdown would have on BuA-induced changes to proliferation and apoptosis.

The key findings from this work included:

(1) Presence of GPCR43 on some but not all CRC cell lines.
(2) Modulation of GPCR43 expression with exposure to BuA and altered glucose concentrations in the media.
(3) An influence of G-protein inhibition on BuA-induced apoptosis but not proliferation in some cell lines.
(4) GPCR43 knockdown using RNAi indicated that GPCR43 is not exclusively required for BuA to regulate apoptosis and proliferation.

The results from this work indicate that GPCR43 is not likely to exclusively mediate BuA's effects, but opens up new areas of research into the exact role of GPCR43 on CRC cells.

Declaration

To the best of my knowledge, this thesis does not contain any material previously submitted for a Degree or Diploma at any University. Material previously written or published by any other person is given due reference in the text.

I give consent to this copy of my thesis, when deposited in the University library, being available for loan and photocopying.

Michelle Zucker
11th February 2008

The difficult is that which can be achieved immediately, the impossible, that which takes a little longer. George Santayana

Acknowledgements

Ok..... I can't believe that this day is actually here! There are a million people to thank for helping me along the way and I fear that this acknowledgement section is going to be one of the longest in history. But here goes….

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And one last thing….. world peace!

Abbreviations

μg	microgram
μl	microlitre
μM	micromolar
°C	degrees Celsius
7TM	seven transmembrane
$A_{260/280}$	ratio of absorbance at 260:280nm
ABI	Applied Biosystems
AOM	azoxymethane
Apaf	apoptotic protease-activating factor 1
APC	Adenomatous polyposis coli
ATP	Adenosine triphosphate
β-Actin	Beta-Actin (gene name)
BCA	Bicinchoninic acid
Block-iT	BLOCK-iT™ Fluorescent Oligo
bp	base pair
BSA	Bovine serum albumin
BuA	butyrate
Ca^{++}	calcium
cAMP	cyclic adenosine monophosphate
cDNA	Complementary DNA
CO_2	carbon dioxide
CRC	colorectal cancer
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CSIRO LI	Commonwealth Scientific and Industrial Research Organisation, Livestock Industries
CSU	Central Services Unit, Adelaide University
Ct	cycling threshold
CV	co-efficient of variance
DAG	diacylglycerol
DEPC	Diethyl pyrocarbonate
DMEM	Dulbecco modified Eagle's minimal essential medium
DMH	dimethylhydrazine
DMSO	Dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNAse	deoxyribonuclease
dNTP	deoxynucleotide triphosphate
dsRNA	double stranded RNA
EDTA	ethylene diamine tetra-acetic acid

EGF	epidermal growth factor
EGF-R	epidermal growth factor receptor
EPIC	European Prospective Investigation in Cancer and Nutrition
F-12	Ham's F-12 nutrient mixture
FA	fatty acid
FACS	fluorescent activated cell sorting
FADD	Fas-associated death domain
FAP	Familial Adenomatous Polyposis
FCS	fetal calf serum
FITC	Fluorescein isothiocyanate
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
gDNA	genomic deoxyribonucleic acid
GDP	Guanosine diphosphate
Glu	glucose
GTP	Guanosine triphosphate
Glut 1	glucose transporter 1
GP2A	G-protein agonist 2A
GPCR	G-protein coupled receptor
H_2O	water
HDAC	histone deacetylase
HPNCC	Hereditary Nonpolyposis Colorectal Cancer
HT29-R	HT29 resistant
IHC	immunohistochemistry
IMVS	Institute of Medical and Veterinary Science
IP_3	Inositol triphosphate
K1	cytokeratin-1
LCMD	laser capture microdissection
MCT1	monocarboxylate transporter 1
$MgCl_2$	magnesium chloride
mL	millilitre
mM	millimolar
mock	mock transfected
MQ	Millique water
mRNA	messenger RNA
N_2	nitrogen
NanoDrop	NanoDrop-1000^{TM}
NEAA	non-essential amino acids
NH_4	ammonium
nm	nanometre
NTC	no template control

OD	optical density
Opti-MEM	Opti-MEM I Reduced-Serum Medium
PARP	poly-(ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PCR	polymerase chain reaction
Pen/Strep	Penicillin/Streptomycin
PIP_2	Phosphatidylinositol bisphosphate
PKC	protein kinase C
PLC	Phospholipase C
PPi	Pyrophosphate
PTP	Protein-Tyrosine Phosphatase
PTX	pertussis toxin
PyMT	polyoma middle T oncogene
Pyr	pyruvate
Q-PCR	Quantitative PCR
RIN	RNA integrity number
RISC	RNA-induced silencing complex
rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute medium
RNA	ribonucleic acid
RNAse	ribonuclease
rRNA	ribosomal RNA
RNAi	RNA interference
RT	reverse transcription
RT-PCR	reverse transcriptase PCR
SCFA	short chain fatty acid
scr	transfected with universal scramble
SD	standard deviation
SEM	standard error of the mean
siRNA	short interfering RNA
SLPI	secretory leukocyte protease inhibitor
SLG1	sodium/glucose co-transporter
SMCT	sodium-coupled monocarboxylate transporter
TAE	tris acetate EDTA
TGF-α	transforming growth factor-α
T_M	melting temperature
tRNA	transfer RNA
TSA	Trichostatin A
untrans	untransfected

U.V.	Ultraviolet
V	volts
VEGF	vascular endothelial growth factor
w/v	weight/volume