



UNIVERSITY OF SYDNEY

APPLICATION OF MAGNETIC RESONANCE  
SPECTROSCOPY  
IN TUMOR PATHOLOGY

by

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## ABSTRACT

High resolution proton magnetic resonance spectroscopy ( $^1\text{H}$  MRS) was used to distinguish between various tumor specimens differing in their malignancy, tumorigenicity, invasiveness and differentiation. This study investigates those MR-visible changes on colorectal carcinoma cells which are specifically due to changes in cellular differentiation.

A poorly differentiated colorectal carcinoma cell line, SW620, was subjected to a differentiation inducer, sodium butyrate (NaBT). 3-5 mM NaBT affected growth rate, morphology, cell cycle phase and activity of brush border marker enzymes indicating increase in cellular differentiation.

Viable butyrate-treated cells were examined by  $^1\text{H}$  MRS. 1-dimensional and 2-dimensional COSY water-suppressed spectra of 3 mM and 5 mM NaBT-treated cells were compared with those of untreated control. One and two dimensional MRS was used to document specific changes in the biochemical profiles of lipid, metabolites and cell surface fucose. NaBT-induced cells can be differentiated from untreated SW620 cells on the basis of their increased MR-visible unsaturated lipid profile, reduced concentration of glycosylation intermediates UDP-hexoses, decreased *N*-acetylation and complexity of cell surface fucosylation pattern. These spectral data were in agreement with those obtained from a more differentiated colorectal cell line, SW1222.

Treatment of cells with NaBT also affected other parameters of culture, not specifically related to cellular differentiation, such as growth phases, sensitivity to mechanical injury, extracellular pH and glucose concentration. In order to distinguish the

effects of these parameters on MR spectra from the changes specifically caused by cellular differentiation, the culture conditions of untreated SW620 were artificially manipulated prior to the MRS examination. These experiments indicated that both increased lipid and reduced UDP-hexoses may not be unique to the process of differentiation, but rather result from environmental changes caused by the treatment.

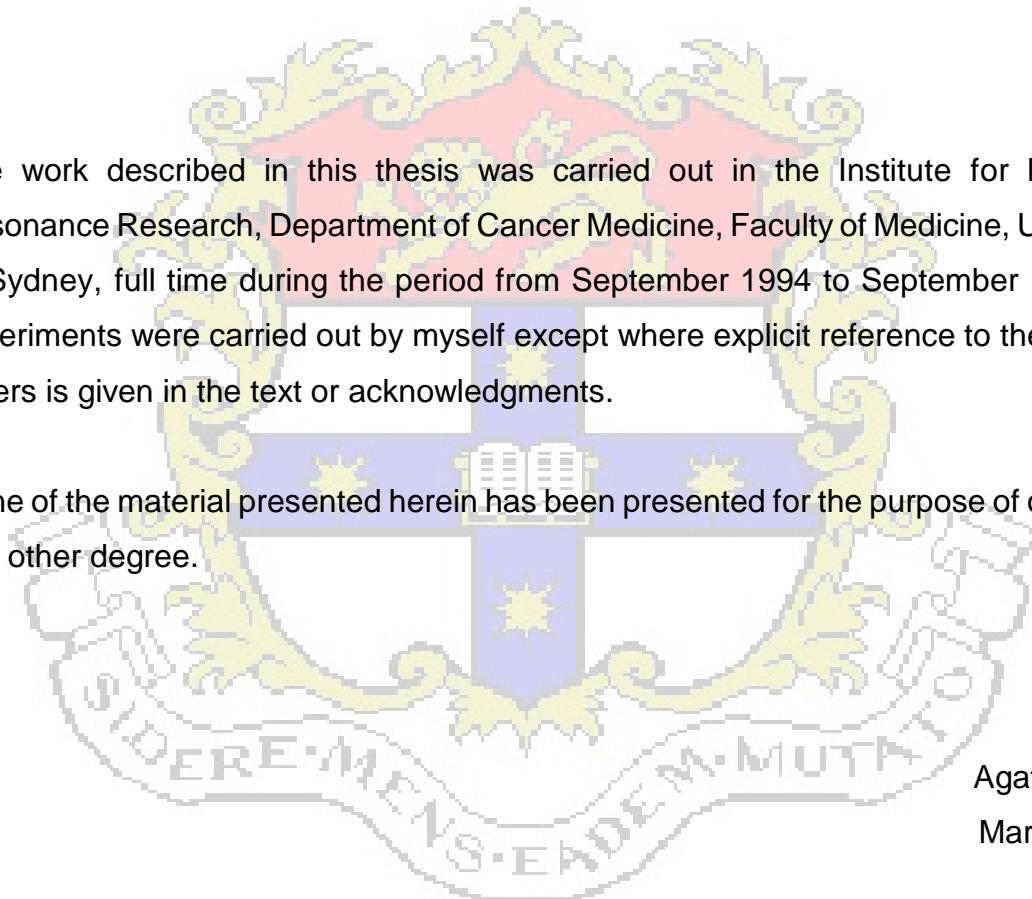
In order to provide further evidence linking differentiation effects with spectral changes, SW620 cells were also subjected to other methods of inducing differentiation, namely treatment with dimethyl sulfoxide, retinoic acid, and replacement of glucose by galactose in the culture media. Cellular differentiation was only obtained after treatment with dimethyl sulfoxide. The results of these experiments confirmed that reduction in MR signals from fucose and *N*-acetylated compounds is associated with differentiated phenotype, while the increase in lipid is not. Additionally, dependence of *N*-acetyl and UDP-hexose concentration on changes in carbohydrate metabolism has been demonstrated.

MRS is able to distinguish colorectal cells according to the degree of their differentiation. These data may contribute to more accurate diagnosis of tumors by distinguishing symptoms of dedifferentiation from those of changes in malignant and metastatic potential. Results presented in this thesis provide chemical evidence in support of the role of differentiation in the well recognised adenoma-carcinoma sequence. MRS also provides biochemical information which, in the future, may aid better understanding of the processes involved in development and progression of cancer.

## STATEMENT OF ORIGINALITY

The work described in this thesis was carried out in the Institute for Magnetic Resonance Research, Department of Cancer Medicine, Faculty of Medicine, University of Sydney, full time during the period from September 1994 to September 1997. All experiments were carried out by myself except where explicit reference to the work of others is given in the text or acknowledgments.

None of the material presented herein has been presented for the purpose of obtaining any other degree.



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## ABBREVIATIONS

1D	one-dimensional
2D	two-dimensional
$^1\text{H}$	proton
$^{12}\text{C}$	carbon 12 nucleus
$^{13}\text{C}$	carbon 13 nucleus
$^{18}\text{O}$	oxygen 18 nucleus
$^{31}\text{P}$	phosphorus 31 nucleus
a.u.	arbitrary units
ATRA	all-trans-retinoic acid
$\mathbf{B}_0$	external magnetic field
$\mathbf{B}_1$	radio frequency magnetic field
cAMP	cyclic adenosine monophosphate
CEA	carcinoembryonic antigen
Cer	ceramide
CH	methine
$\text{CH}_2$	methylene
$\text{CH}_3$	methyl
CH=CH	olefinic carbons
Cho	choline
Cho-P	phosphocholine
CMP	citidine monophosphate
$\text{CO}_2$	carbon dioxide
COSY	COrelated SpectroscopY (two-dimensional scalar)
CPMG	Carr-Purcell-Meiboom-Gill (pulse sequence)
CRC	colorectal cancer
CX-1	colorectal carcinoma cell line
dB	decibels
$\text{D}_2\text{O}$	deuterium oxide (heavy water)
DMEM	Dulbecco's Modified Eagle Medium
DMSO	dimethyl sulfoxide
E	energy
EDTA	ethylene diamine tetra-acetic acid

Etn	ethanolamine
FBS	fetal bovine serum
$F_1$	frequency in the first dimension of a 2D experiment
$F_2$	frequency in the second dimension of a 2D experiment
FID	free induction decay
Fuc (I, II, III)	fucose
FT	Fourier transformation
$G_1, G_2$	cell cycle phases
Gal	galactose
GalNAc	<i>N</i> -acetyl-galactosamine
Glc	glucose
Glc <sup>-</sup>	glucose-free
Glc <sup>-</sup> /Gal <sup>+</sup>	substitution of glucose with galactose in the medium
GB	Gaussian broadening
GDP	guanidine di-phosphate
GlcNAc	<i>N</i> -acetyl-glucosamine
GPC	glycero-phosphocholine
Gz	gradient magnetic field in z direction
h	hour
$\hbar$	Planck's constant
H <sub>2</sub> O	water
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
H <sub>2</sub> SO <sub>4</sub>	sulfuric acid
Hx	hydrogens attached to carbon C <sub>x</sub> of carbohydrate moieties
HT-29	colorectal carcinoma cell line
HRT-18	colorectal carcinoma cell line
Hz	hertz
I	nuclear spin
Ins	inositol
LAOR	L-amino-oxidase reagent
LB	Lorentzian broadening
LDH	lactate dehydrogenase
Le	Lewis antigen, (Le <sup>a</sup> , Le <sup>b</sup> , Le <sup>y</sup> , Le <sup>x</sup> )
Lys	lysine

M	mole; mitotic cell cycle phase
<b>M</b>	magnetization vector
MAb	monoclonal antibody
MHz	megahertz
min.	minute
mM	millimole
MR	magnetic resonance
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
ms	millisecond
mm	millimeter
n2T	delay period in 1D T <sub>2</sub> -filtered MR experiments where n - number
of	π pulses, t=1ms
n	number of experiments
NaBT	sodium butyrate
NANA, Neu5Ac	<i>N</i> -acetyl neuraminic (sialic) acid
NAc	<i>N</i> -acetyl
ND	not done
NE	number of experiments
<sup>+</sup> N(CH <sub>3</sub> ) <sub>3</sub>	<i>N</i> -trimethyl
NMR	nuclear magnetic resonance
NS	number of scans
O1	offset
PBS	phosphate buffered saline
PCA	perchloric acid
PCR	polymerase chain reaction
PCr	phosphocreatine
p	in T-test probability that two means are equal
PLC	phospholipase C
ppm	parts per million
RA	retinoic acid
Rib	ribose
r.f.	radio frequency
rpm	revolutions per minute



S	cell cycle phase (synthesis)
S.D.	standard deviation
SW	sweep width
SW1222	colorectal carcinoma cell line
SW620	colorectal carcinoma cell line
$t_1$	time domain in the first dimension
$t_2$	time domain in the second dimension
$t_d$	doubling time of cell culture
$T_1$	spin-lattice relaxation (longitudinal)
$T_2$	spin-spin relaxation (transverse)
TGF- $\beta$	transforming growth factor $\beta$
Thr	threonine
Tn	cell surface glycoprotein antigen
UDP	uridine diphosphate
Ura	uracil
x,y,z	x, y and z coordinate axes
$\alpha,\beta$	anomer conformations of carbohydrate molecules or glycosidic bonds
$\gamma$	gyromagnetic ratio
$\gamma$ GT	$\gamma$ -glutamyl transpeptidase
$\delta$	chemical shift
$\mu$	nuclear magnetic moment
$\mu\text{m}$	micrometers
$\nu$	frequency
$\nu_{1/2}$	linewidth at half height
$\omega, \omega_0$	Larmor frequency

## TABLE OF CONTENTS

	Page
CHAPTER 1: INTRODUCTION .....	1
CHAPTER 2: MATERIALS AND METHODS .....	41
CHAPTER 3: SODIUM BUTYRATE INDUCED DIFFERENTIATION OF SW620 HUMAN MALIGNANT COLORECTAL CELLS .....	66
CHAPTER 4: <sup>1</sup> H MR SPECTRA OF COLORECTAL CARCINOMA SW620 CELL LINE CHANGE WITH NaBT-INDUCED DIFFERENTIATION .....	95
CHAPTER 5: EFFECTS OF NaBT-INDUCED CULTURE CONDITIONS ON MR SPECTRA OF CELLS .....	150
CHAPTER 6: CHARACTERIZATIONS OF OTHER POTENTIAL DIFFERENTIATION-INDUCING AGENTS .....	180
CHAPTER 7: CONCLUSIONS .....	265
REFERENCES .....	271
APPENDIX I: DETERMINATION OF DOUBLING TIME .....	298
APPENDIX II: PROTON MAGNETIC RESONANCE SPECTROSCOPY .....	302

# LIST OF FIGURES

## CHAPTER 1

page

- 1.1. Chemical structures of some glycosylated antigens associated with colorectal cancers . . . . . 27
- 1.2. Some molecules and chemical groups observed to change in colorectal cancer progression . . . . . 39

## CHAPTER 2

- 2.1. Colorectal carcinoma cell lines *in vitro*: SW620 (poorly differentiated) and SW1222 (moderately differentiated). . . . . 43

## CHAPTER 3

- 3.1. Chemical structure of sodium butyrate . . . . . 67
- 3.2. Short-term growth curve of human malignant colorectal cells (SW620) exposed to sodium butyrate at different concentrations: 0 - 5mM . . . . . 70
- 3.3. Long-term growth curves of adhered SW620 cells grown in medium containing 3 mM and 5 mM sodium butyrate. . . . . 71
- 3.4. Percentage of cell cycle phases in SW620 after treatment with 3 mM sodium butyrate . . . . . 74
- 3.5. Sodium butyrate - treated SW620 cells: Contrast phase micrographs . . . . . 76
- 3.6. Extracellular pH changes vs NaBT concentration. . . . . 83
- 3.7. Glucose consumption vs concentration of NaBT . . . . . 85

## CHAPTER 4

- 4.1. SW620 cells (control): 360 MHz <sup>1</sup>H MR 1D and COSY . . . . . 99
- 4.2. Sodium butyrate effect: 360 MHz <sup>1</sup>H MR 1D spectra of SW620 cells treated with 3 mM and 5 mM NaBT for 72 h . . . . . 107
- 4.3. NaBT-treatment: 360 MHz <sup>1</sup>H MR COSY spectrum of SW620 cells treated with 3 mM and 5 mM NaBT for 72 h . . . . . 110-112

4.4. Sodium butyrate effect: 360 MHz $T_2$ - filtered 1D spectra of SW620 cells treated with 5 mM NaBT for 72 h	115
4.5. Sodium butyrate effect: 360 MHz $^1\text{H}$ MR symmetrized COSY spectra showing methyl - methine coupling region	117
4.6. Reversal of NaBT effect: 360 MHz $^1\text{H}$ MR 1D spectra of SW620 cells pre-treated with 3 mM NaBT for 72 hours and then cultured without NaBT for: 0, 2, 3, 6, 10, or 17 days.	120
4.7. Cross-peak volume ratios for total fucose, UDP-hexoses and lipid cross-peak B to Lys from the spectra of cells pre-treated 3 days with 3 mM NaBT and subsequently cultured in standard medium	121
4.8. SW1222 compared to SW620 cell line: 360 MHz $^1\text{H}$ MR standard and $T_2$ - filtered 1D spectra	125
4.9. SW1222 cell line: 360 MHz $^1\text{H}$ MR symmetrized COSY spectrum	126-127
4.10. SW1222 cell line: 360 MHz $^1\text{H}$ MR symmetrized COSY spectra showing methyl-methine coupling region	130
4.11. Comparison of cross-peak volumes: lipid cross-peak, total fucose block, ribose (UDP) and lipid cross-peak ratio D/C in SW620, treated with 0, 3 and 5 mM NaBT, and in untreated SW1222 cell lines.	133

## CHAPTER 5

5.1. Schematic comparison of growth curves of control SW620 and treated with butyric acid.	152
5.2. 0% viable (frozen) SW620 cells: 360 MHz $^1\text{H}$ MR 1D spectra	156
5.3. SW620 cells control and "frozen": 360 MHz $^1\text{H}$ MR COSY	159-160
5.4. 0% viable (frozen) SW620 cells: 360 MHz $^1\text{H}$ MR symmetrized COSY spectra showing methyl - methine coupling region	162
5.5 $^1\text{H}$ MR spectral changes as function of extracellular pH in SW620	163
5.6. Glucose concentration effect: 360 MHz $^1\text{H}$ MR 1D spectra of SW620 cells grown in medium containing: 0 mM, 25 mM, or 40 mM glucose.	166
5.7. Glucose concentration effect: $^1\text{H}$ MR COSY spectra showing methyl - methine coupling region of SW620 cells grown in 0-40 mM glucose.	168
5.8. Glucose dependence of proton MRS signals	169

## CHAPTER 6

6.1. Chemical structure of dimethyl sulfoxide. . . . .	181
6.2. Chemical structure of retinoic acid . . . . .	183
6.3 Growth curves of SW620 cells treated with 2% and 3% dimethyl sulfoxide	186
6.4. DMSO - treated SW620 cells: Contrast phase micrographs . . . . .	188
6.5. Cell cycle phase profile of control SW620 and 3% DMSO-treated cells . . . .	190
6.6. Dimethyl sulfoxide effect: 360 MHz $^1\text{H}$ MR 1D spectra of SW620 treated with 2% and 3% DMSO for 72 h or 14 days . . . . .	193
6.7. Dimethyl sulfoxide effect: $T_2$ - filtered 1D spectra . . . . .	195
6.8. DMSO-treated SW620 cells: 360 MHz $^1\text{H}$ MR COSY spectra . . . . .	197-200
6.9. DMSO effect: 360 MHz $^1\text{H}$ MR symmetrized COSY spectra showing methyl - methine coupling region . . . . .	202
6.10. Growth curves of SW620 cells treated with $3.3 \times 10^{-5}$ M and $4.95 \times 10^{-5}$ M retinoic acid . . . . .	203
6.11. Retinoic acid - treated SW620 cells: Contrast phase micrographs . . . . .	205
6.12. Cell cycle phase profiles after treatment of SW620 cells with $4.59 \times 10^{-5}$ M and $6.6 \times 10^{-5}$ M retinoic acid . . . . .	206
6.13. Retinoic acid effect: 360 MHz $^1\text{H}$ MR 1D spectra of SW620 cells treated with $3.3 \times 10^{-5}$ M - $6.6 \times 10^{-5}$ M ATRA for 72 h . . . . .	209
6.14. Retinoic acid effect: 360 MHz $^1\text{H}$ MR COSY spectra . . . . .	213-216
6.15. Suspension culture: 360 MHz $^1\text{H}$ MR 1D spectra of SW620 cells grown for 72 hours in suspension phase . . . . .	220
6.16. Suspension culture: 360 MHz $^1\text{H}$ MR COSY spectra of cells grown for 72 hours in suspension phase . . . . .	222
6.17. Growth curve of adhered SW620 cells cultured to confluency in glucose-free / galactose-enriched medium. . . . .	225
6.18. Long-term growth curve of SW620 grown in glucose-free/galactose-enriched medium. . . . .	226
6.19. Glc - depleted / Gal - enriched SW620 cells: Contrast phase micrographs	228
6.20. Distribution of cell cycle phases in SW620 grown in glucose-free/galactose- enriched medium . . . . .	229
6.21. Glucose depletion/galactose enrichment effect: 360 MHz $^1\text{H}$ MR 1D spectra of SW620 cells grown in glucose free / galactose enriched medium for 72h.	232
6.22. Glc - depletion / Gal - enrichment effect: 360 MHz $^1\text{H}$ MR COSY spectrum	

of SW620 cells grown in glucose free/galactose enriched medium for 72 h .....	234-235
6.23. Glucose to galactose substitution effect: 360 MHz $^1\text{H}$ MR symmetrized COSY spectra (NS=32, NE=200) showing methyl - methine coupling region . . . .	238

## LIST OF TABLES

### CHAPTER 3

3.1. Doubling times calculated for subsequent successive approximation steps of control SW620 cells and 3 mM NaBT-treated based on assumption that all cells proliferate at a constant rate . . . . .	73
3.2. Percentage of adhered (monolayer) SW620 cells and their viability after treatment with 0-5mM NaBT . . . . .	79
3.3. Marker enzyme activities in sodium butyrate treated SW620 cells . . . . .	80
3.4. Total protein concentration (ng/cell) in control cells and treated with 3 mM and 5 mM sodium butyrate. . . . .	81
3.5. Concentration of glucose in the media collected from cell cultures after 48 hours of treatment with sodium butyrate. . . . .	84

### CHAPTER 4

4.1. Assignment of major resonances in 1D proton MR spectra of SW620 highly tumorigenic cultured CRC cell lines. . . . .	103
4.2. Major chemical species identified in 2D <sup>1</sup> H MR spectra of human CRC cell lines. . . . .	104
4.3. Viability of cells harvested from early confluent monolayer by scraping with a NUNC cell scraper . . . . .	105
4.4. 1D spectral changes occurring during NaBT-induced cellular differentiation. . . . .	108
4.5. 2D spectral changes in lipid carbohydrate and amino acid cross-peaks after NaBT-induced differentiation . . . . .	113
4.6. Summary of SW620 cell characteristics after 72-hour culture in NaBT containing medium . . . . .	123

### CHAPTER 5

5.1. 1D peak height ratios in the spectra of untreated SW620 cells at 3 different stages of culture. . . . .	154
5.2. Cross-peak volume ratios in the spectra of untreated SW620 cells at 3 different stages of culture. . . . .	154

5.3. 1D - spectral changes occurring in cells after freezing. . . . .	157
5.4. 2D - spectral changes occurring in cells after freezing. . . . .	161

## CHAPTER 6

6.1. Marker enzyme activities of SW620 cells grown in 2% and 3% DMSO for 3 days. . . . .	191
6.2. 1D spectral changes occurring during DMSO-induced cellular differentiation . . . . .	194
6.3. 2D spectral changes in SW620 cells after DMSO-induced differentiation. . .	201
6.4. Extracellular pH values depending on the concentration of retinoic acid . . .	206
6.5. Marker enzyme activities of cells grown in retinoic acid . . . . .	207
6.6. 1D spectral changes occurring after treatment with retinoic acid . . . . .	211
6.7. 2D spectral changes occurring after treatment of cells with retinoic acid. . . .	217
6.8. 2D - spectral changes occurring when SW620 cells are growing in suspension phase. . . . .	221
6.9. Marker enzyme activities of SW620 grown in glucose-depleted galactose-containing medium . . . . .	230
6.10. 1D spectral changes occurring in SW620 cells grown on galactose instead of glucose . . . . .	233
6.11. 2D - spectral changes occurring during glucose depletion/galactose-enrichment. . . . .	237
6.12. Summary of SW620 cell characteristics after culture in DMSO, retinoic and glucose-instead-of-galactose containing medium . . . . .	239