UNIVERSITY OF SYDNEY

APPLICATION OF MAGNETIC RESONANCE SPECTROSCOPY IN TUMOR PATHOLOGY

by

Agata Rekas

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ABSTRACT

High resolution proton magnetic resonance spectroscopy (^{1}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}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}H	proton
^{12}C	carbon 12 nucleus
^{13}C	carbon 13 nucleus
^{18}O	oxygen 18 nucleus
^{31}P	phosphorus 31 nucleus
a.u.	arbitrary units
ATRA	all-trans-retinoic acid
$\mathbf{B_o}$	external magnetic field
$\mathbf{B_1}$	radio frequency magnetic field
cAMP	cyclic adenosine monophosphate
CEA	carcinoembryonic antigen
Cer	ceramide
CH	methine
CH_2	methylene
CH_3	methyl
CH=CH	olefinic carbons
Cho	choline
Cho-P	phosphocholine
CMP	citidine monophosphate
CO_2	carbon dioxide
COSY	COrrelated SpectroscopY (two-dimensional scalar)
CPMG	Carr-Purcell-Meiboom-Gill (pulse sequence)
CRC	colorectal cancer
CX-1	colorectal carcinoma cell line
dB	decibels
D_2O	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
Gal*N*Ac	*N*-acetyl-galactosamine
Glc	glucose
Glc^-	glucose-free
Glc^-/Gal^+	substitution of glucose with galactose in the medium
GB	Gaussian broadening
GDP	guanidine di-phosphate
Glc*N*Ac	*N*-acetyl-glucosamine
GPC	glycero-phosphocholine
Gz	gradient magnetic field in z direction
h	hour
$\hbar$	Planck's constant
H_2O	water
H_2O_2	hydrogen peroxide
H_2SO_4	sulfuric acid
Hx	hydrogens attached to carbon Cx 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^a, Le^b, Le^y, Le^x)
Lys	lysine

M	mole; mitotic cell cycle phase
M	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
n2τ	delay period in 1D T_2-filtered MR experiments where n - number
of	π pulses, t=1ms
n	number of experiments
NaBT	sodium butyrate
NANA, Neu5Ac	*N*-acetyl neuraminic (sialic) acid
*N*Ac	*N*-acetyl
ND	not done
NE	number of experiments
$^{+}N(CH_3)_3$	*N*-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-latice relaxation (longitudinal)
T_2	spin-spin relaxation (transverse)
TGF-β	transforming growth factor β
Thr	threonine
Tn	cell surface glycoprotein antigen
UDP	uridine diphosphate
Ura	uracil
x,y,z	x, y and z coordinate axes
α,β	anomer conformations of carbohydrate molecules or glycosidic bonds
γ	gyromagnetic ratio
γGT	γ-glutamyl transpeptidase
δ	chemical shift
μ	nuclear magnetic moment
μm	micrometers
ν	frequency
$\nu_{1/2}$	linewidth at half height
ω, ω_o	Larmor frequency

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