Epigenetic Characterisation of the O6 Methyl-Guanine DNA-Methyltransferase Promoter in New Zealand Melanoma Cell Lines

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Abstract

New Zealand has the second highest incidence of melanoma skin cancer in the world. Chemotherapy is the standard treatment for melanoma derived tumours which have undergone metastasis and current therapies have limited benefit. There is a great need for new therapies and to increase the efficacy of current therapies.

Temozolomide (TMZ) is a chemotherapy agent effective in the treatment of both metastatic melanoma and glioblastoma (brain cancer), although TMZ resistance has been observed in many tumours. The activity of the DNA repair enzyme O^6 methyl-guanine methyltransferase (MGMT) is thought to be largely responsible for TMZ resistance.

MGMT protects the cell from the effects of TMZ by removing cytotoxic lesions placed on the DNA. Mechanisms of regulation of MGMT expression remain unclear in melanoma. DNA methylation at the MGMT promoter has been linked to MGMT silencing in some cancers and has been associated with specific chromatin modifications. The present study was aimed at investigating the promoter methylation status of MGMT in primary melanoma cell lines using a new technique named methyl DNA immuno-precipitation (MeDIP). Next, the chromatin immuno-precipitation (ChIP) method was used to examine post translational modifications on the surrounding chromatin. The data obtained was correlated with both MGMT transcription levels and TMZ sensitivity.

The promoter methylation status of MGMT has been used to predict the clinical responsiveness of glioblastoma patients to TMZ. Establishing the regulatory mechanisms of MGMT expression in melanoma patients would validate a means to predict clinical responsiveness to TMZ. Furthermore, establishing mechanisms of MGMT silencing may provide the basis for future clinical trials of novel therapies for melanoma and glioblastoma.

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Abbreviations

°C	degrees celsius
ADP	adenosine 5’-diphosphate
AGT	alkylguanine-transferase
AGAT	alkylguanine-alkyltransferase
AP1	activator protein 1
AP2	activator protein 2
ATP	adenosine 5’-triphosphate
ATF	activating transcription factor
α-MEM	alpha-minimal essential medium
bp	base pair
cAMP	cyclic adenosine 5’-monophosphate
CBP	cAMP response element binding protein
CHD	chromodomain helicase DNA-binding chromatin remodelling complex
ChIP	chromatin immunoprecipitation
Cp	crossing point
CpG	cytosine-guanine dinucleotide
CTD	carboxyl terminal domain
dNTP	2’-deoxynucleotide 5’-triphosphate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNMT	DNA methyl-transferase
ds	double stranded
DSB	double stranded break
EDTA	ethylene-diamine-tetra-acetic acid
EJ	end joining
ES	embryonic stem cell
EtBr	ethidium bromide
FACT	facilitates chromatin transcription
FBS	foetal bovine serum
g	gram
gDNA	genomic DNA
GR	glucocorticoid receptor

GRE	glucocorticoid response element
H3K9	lysine 9 of histone H3
H3K9ac	acetylated lysine 9 of histone H3
H3K9me2	dimethylated lysine 9 of histone H3
H3K9me3	trimethylated lysine 9 of histone H3
HAT	histone acetyl transferase
HCl	hydrochloric acid
HDAC	histone deacetylase
HDACi	histone deacetylase inhibitor
HKMT	histone lysine methyl transferase
HP1	heterochromatin protein 1
HR	homologous recombination
INO80	inositol family chromatin remodelling complex
IP buffer	immunoprecipitation buffer
ISWI	imitation switch family chromatin remodelling complex
ITS	insulin-transferrin selenite
JmjC	jumanji-C domain
kb	kilobase-pairs
L	litre
LC480	lightcycler 480 instrument
LSD	lysine demethylase
M	moles per litre
MAF	masculoaponeurotic fibrosarcoma
MBD	methyl binding domain
MeCP2	methyl CpG binding protein 2
MeDIP	methyl-DNA immunoprecipitation
MGMT	O6 methyl-guanine DNA-methyltransferase
µg	microgram
µL	micolitre
µM	micro moles per litre
mg	milligram
mL	millilitre
MML	mixed lineage leukemia
mM	milli moles per litre

MMR	mismatch repair
M_r	relative molecular mass (g mol^{-1})
mRNA	messenger ribonucleic acid
MTIC	5-(3-methyltriazen-1-yl)imidazole-4-carboxamide
NaOAc	sodium acetate
ncRNA	non-coding RNA
NDR	nucleosome depleted region
Nmol	nanomole
Nt	nucleotide
NZM	New Zealand melanoma
O^6-MG	guanine nucleotide with methylation of the O^6 position
OGAT	O^6-alkylguanine transferase
PBS	phosphate buffered saline
PcG	polycomb group
PCR	polymerase chain reaction
pH	-Log $[H^+]$
PKC	protein kinase C
PRMT	protein arginine methyltransferase
RB	retinoblastoma protein
RISC	RNA-induced silencing complex
RITS	RNA-induced transcriptional silencing
RNA	ribonucleic acid
RNApolII	RNA polymerase II
RNApolIIo	elongation competent RNApolII
RNase	ribonuclease
RNAi	RNA interference
ROS	reactive oxygen species
rpm	revolutions per minute
RT	room temperature
RT-qPCR	real time-quantitative polymerase chain reaction
s	second
SAM	s-adenosyl methionine
SDS	sodium dodecyl sulphate
siRNA	short interfering RNA

SWI/SNF	mating type switching/sucrose non-fermenting family
TAE	tris-HCl, acetic acid, EDTA buffer
TBP	TATA binding protein
TE	tris-HCl, EDTA buffer
TF	transcription factor
TMZ	temozolomide
Tris	tris(hydroxymethyl)aminomethane
TrypleE	express stable trypsin-like enzyme plus phenol red
TSP	transcription start point
UV	ultraviolet light
V	volt (m^2 kg s^{-3} A^{-1})