Protein Interactions at the Human Topoisomerase IIα Promoter

A thesis presented to Massey University in partial fulfilment of the requirement for the degree Doctor of Philosophy in Biochemistry.

Natisha Magan

2009

If these written words are an indication of
what I have learnt and who I have become……
then let a blank page represent
what I have yet to learn and who I am yet to become……

Acknowledgements

Many, many, many, many thanks to my wonderful supervisor Associate Professor Dr. Kathryn Stowell. Thank you for all your help, your guidance, your honesty, your support, your time and enthusiasm. I have truly enjoyed my time in your lab, I am fortunate to have had the chance to work with a group of talented, brilliant and beautiful people over the years, thank you for that opportunity.

I would like to thank Massey University for the financial support of a Doctoral scholarship. I would have never been able to take on such a task nor survive the extended period without additional financial support from The Todd Foundation, as well as the Edward and Isobel Kidson scholarship. It was greatly appreciated, thank you.

I am not going to list all the people that have helped me. There are just far too many of you. My friends, my family, my colleagues, all of you have influenced my life and helped me in so many different ways. I am incredibly lucky to have so many people who look out for me, who are there to support me, to guide me, who make me laugh and see a brighter day. I appreciate all of you, I truly, truly do.

Mum, who through it all, believes in me the most. It is because of you, I always try my best. It is because of you, I have learnt my strengths. It is because of you, I'm not afraid to fall…because I know, you will always catch me if I do. Thank you for just being mum. Finally, Dad….your Natsu has finally done it. I wish you were here to see this one.

Abstract

Among women in the 45 to 64 age group, over half of the recorded deaths are from cancer, breast cancer being the most common. Just over 30% off all deaths in New Zealand women is caused by breast cancer. Treatment of cancer is difficult, not only due to the physiological and immunological similarities between a cancer cell and a normal cell, but also due to the high cardiotoxicity of many treatments, and also the problems related with the development of resistance. Approximately 40% of the cancer cells treated with the chemotherapy drug doxorubicin will become resistant to treatment. Drug efficacy is strongly associated with the proliferation status of a cell, as cancer cells divide rapidly, this can often be the defining factor between effective treatments or the development of resistance. Central to this proliferation status is an enzyme known as topoisomerase IIα. This essential enzyme is expressed in all cells and is required to relieve the torsional stress in DNA that is created during normal cellular processes. A number of commonly used anti-cancer drugs have been found to target topoisomerase IIα in cancer cells and significantly, during the development of drug resistance levels of topoisomerase IIα enzyme have been found to be reduced in some cell lines and tumours. There are a number of factors that can modulate the amount of topoisomerase IIα enzyme found in a cell, and one of the ways to understand this is to examine the regulation of the topoisomerase IIα gene, most importantly the proteins that interact with the promoter region to direct transcription.

The human topoisomerase IIα promoter has been found to be regulated by a number of transcription factors that can bind to their cognate sequences. The introduction of mutations within specific sequences of the topoisomerase IIα promoter has enabled the identification of a key regulatory region within the promoter, a sequence of DNA that encompasses both the ICB1 and GC1 regulatory elements. Transcription factor NF-Y is found to bind to ICB1 element, whereas transcription factors Sp1 and Sp3 have been found to associate with the GC element. However this region of the promoter was also found to bind a fourth uncharacterised component. This research aims to further define the protein components that are found to bind to this important ICB1/GC1 regulatory region and distinguish the protein-protein and protein-DNA interactions that are important for the regulation of the human topoisomerase IIα promoter.

Abbreviations.

1^o	Primary
2^o	Secondary
APAF	Australian Proteome Analysis Facility
APS	Ammonium persulphate
AP-2	Activator protein 2
ATF	Activating transcription factor
ATP	Adenosine triphosphate
bp	Base Pair
β-gal	β-galactosidase
BrdU	Bromodeoxyuridine
BSA	Bovine serum albumin
Buffer E	Equilibration buffer
CDE	Cell-cycle dependent element
CDTA	1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid
CEM	Human leukemic cell line
CENins	ICB1/GC1 altered to have an inserted sequence within the intervening region
CENdel	ICB1/GC1 altered to have a deletion within the intervening region
CENmt	ICB1/GC1 altered to have a mutation within the intervening region
ChIP	Chromatin immunoprecipitation
CHR	Cell-cycle gene homology region
COS-1	SV40 transformed African green monkey kidney cells
ddH_2O	Double distilled water
DNA	Deoxyribose Nucleic Acid
DNA-PK	DNA-dependant protein kinase
DNA-PKcs	DNA-dependant protein kinase catalytic subunit
dNTP	Deoxyribonucleotide triphosphate
DSB	DNA double-strand break
DTT	dithiothreitol
EDTA	Ethylene diamine tetra-acetic acid
EMSA	Electrophoretic Mobility Shift Assay
GCF	GC rich sequence binding factor

GSB	Gel shift buffer
GZP1	GC box-binding protein-1
H209/VP	Human small-cell lung cancer cell line resistant to etoposide (VP-16)
HBT20	Human brain tumour cell line
HCl	Hydrochloric acid
HeLa	Human cervical cancer cell line
HEPES	N-[2-hydroxyethyl]piperazine-N'-[2-ethane sulfonic acid]
Her-2	Human epidermal growth factor 2
HL-60	Human promyelocytic leukemia cells
HL-60/MX2	Human promyelocytic leukemia cells resistant to mitoxantrone
HMGB	High mobility group B proteins
HRP	Horse-radish peroxidase
ICB	Inverted CCAAT box
ICBP90	Inverted CCAAT box binding protein of 90 kDa
IgG	Immunoglobulin G
IPTG	Isopropyl thiogalactosidase
KB	Human epidermoid cancer cell line
KCl	Potassium chloride
kDa	Kilo Dalton
Ku86	Ku autoantigen 86
Ku70	Ku autoantigen 70
Ku	heterodimeric protein consisting of Ku86 and Ku70
LB	Luria Bertani bacteriological media
MCF7	Human breast cancer cells
MCF12A	Normal human breast cells
mt	Mutant
MgAc	Magnesium acetate
Mw	Molecular Weight
NaCl	Sodium chloride
NIH3T3	Cells established from NIH swiss mouse embryo, highly contact inhibited
PARP	Poly (ADP-ribose) polymerase
PBS	Phosphate buffered saline solution
PCR	Polymerase chain reaction
pGL3B	pGL3B vector (for luciferase reporter gene assays)

PMSF	Phenylsulfonylmethyl fluoride
PSF	Polypyrimidine tract binding protein
PVDF	Polyvinylidene fluoride
NF-Y	Nuclear factor Y
mM	milli molar
µL	Micro Litre
µg	Micro Gram
NF90	Nuclear family 90
ng	nano gram
pRb	retinoblastoma protein
RNA pol II	RNA polymerase II
SDS-PAGE	Sodium dodecylsulphate polyacrylamide gel electrophoresis
Sp1	Specificity factor 1
SUMO	Small ubiquitin-like modifier
SV40	Simian virus 40
TBE	Tris borate EDTA
TBP	TATA-binding protein
TBST	Tris-buffered saline tween-20
TE	Tris-EDTA buffer
TEMED	Tetramethylethylenediamine
Topo2α	Topoisomerase IIα
Topo2β	Topoisomerase IIβ
TPA	12-O-tetradecanoylphorbol-13-acetate
Tris	Tris (2-amino-2-hydromethyl-1,3-propandiol)
TSS	Transcription start site
U937	Human monocytic leukaemia cell line
UV	Ultra Violet
V	Volts
VM-26	Teniposide
VP-16	Etoposide
wt	Wild type
X-gal	5-bromo-4-chloro-3-indoyl-β-D-galactosidase.
ZBP89	Zinc finger binding protein of 89 kDa

List of Figures

List of Tables

Table of Contents