An investigation into the regulation of the topoisomerase IIα promoter in breast cancer cells exposed to doxorubicin

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

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2003

Dedication

This thesis is dedicated to my parents, Gary and Sandra Allen, and to my twin sister Kim Allen for all their support and encouragement over the last four years.

Acknowledgments

I would like to thank my supervisors Kathryn Stowell, Richard Isaacs and John Tweedie for all their expertise, enthusiasm and support that made this study possible. I would like to also thank the occupants of the Twilight Zone especially Carole Flyger, Robyn Marston, Natisha Magan and Chrissie Down for their patience and tolerance.

I acknowledge the financial support of the New Zealand Cancer Society, the Taranaki Branch of the Cancer Society, the Goodman Family Trust and the Institute of Molecular BioSciences, Massey University that made this research possible.

Abstract

Chemotherapeutic drugs, such as doxorubicin, are some of the most effective agents for the treatment of breast cancer. Acquired resistance to these drugs often develops, however, and may preclude effective treatment. Such resistance is multifactorial in origin, but may include down-regulation of topoisomerase IIα (topo IIα) - an essential enzyme involved in normal DNA metabolism and a target for some of the anticancer drugs. A reduction in the levels of this enzyme is thought to reduce DNA damage induced by the drug-topo IIα complex and so increases the chances of survival.

The mechanisms involved in this down-regulation and the development of resistance to doxorubicin are the focus of this study. Stable breast cancer cell lines, containing deletion constructs of the topo IIα promoter linked to the hGH reporter gene, were exposed to doxorubicin and both the reporter and endogenous gene expression were analysed in the surviving cells. It was shown that the reporter and endogenous topo IIα gene expression in the cell line containing the full length topo IIα promoter construct was no longer correlated in the surviving cells negating the use of this experimental system. Instead the endogenous expression of topo IIα and putative regulatory factors were investigated.

Data suggest that specific cell lines show a down-regulation in the levels of the topo IIα protein. These changes were not due to changes in cellular proliferation rates, cell cycle profile or promoter sequence. Selected cell lines were analysed for changes in the relative amounts of specific transcription factors with putative roles in topo IIα gene regulation and for the expression of proteins proposed to have a role in the development of drug resistance. In specific cell lines, a reduction in topo IIα protein levels correlated with alterations in the relative amounts of NF-YA and/or Sp1. It was shown that the drug efflux pumps MDR1 and MRP1, as well as the heat shock factor Hsp70 were not involved in the survival of cells that were exposed to the drug. *In vivo* footprinting was attempted to detect changes in the *in vivo* binding of proteins to the topo IIα promoter after short term drug exposure.

Abbreviations

A	adenine
a.a	amino acid
AC	adenylyl cyclase
AP-1-4	activator protein-1-4
ATF	activating transcription factor
ATP	adenosine triphosphate
bp	base pair
BSA	bovine serum albumin
C	cytosine
CaCO-2	human colon carcinoma cell line
cAMP	cyclic adenosine monophosphate
CAPS	3-(cyclohexylamino)-1-propanesulfonic acid
cDNA	complementary DNA
CDK1	cyclin-dependent kinase 1
CDP	CCAAT displacement protein
C/EBP	CCAAT enhancer binding protein
CEM	human leukemic cell line
CKII	casein kinase II
cpm	counts per minute
C-terminal	carboxyl terminal
CTF (NF-1)	CCAAT transcription factor
CTP	cytosine triphosphate
DAG	diacylglycerol
DBD	DNA-binding domain
DEPC	diethyl pyrocarbonate
DMSO	dimethyl sulphoxide
DNase I	deoxyribonuclease I
dNTPs	deoxynucleoside triphosphates
dox^R	doxorubicin-resistant
DTT	dithiothreitol

E. coli	*Escherichia coli*
EDTA	ethylene diamine tetra-acetate
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
Egr-1	early growth response protein-1
ELISA	enzyme-linked immunosorbent assay
ER	estrogen receptor
ERE	estrogen response element
ERK	extracellular signal-regulated kinase
FACS	fluorescence activated cell sorting
FCS	fetal calf serum
G	guanine
G418	neomycin sulphate
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GCG	Genetics Computing Group
GRE	glucocorticoid responsive element
GTP	guanosine triphosphate
HAT	histone acetyltransferase
HeLa	human cervical carcinoma cell line
HEPES	N-2-hydroxyethyl piperazine-N'-2-ethane sulfonic acid
HepG2	human hepatocarcinoma cell line
HFM	histone fold motif
hGH	human growth hormone
HL-60	human promyelocytic leukemia cell line
hr	hour
hRPB11/13	human RNA polymerase II subunit 11/13
HSE	heat shock element
HSF	heat shock factor
Hsp70	Heat shock protein 70
HSP-CBF	Heat shock protein - CCAAT binding protein
ICB	inverted CCAAT box
ICB90	ICB binding protein 90

IgG	immunoglobulin G
IP3	inositol triphosphate
JNK	jun N-terminal kinase
JNKK	JNK kinase
K562	human erythroleukemic cell line
kb	kilobase
LMP	ligation-mediated PCR
MAPK	mitogen-activated protein kinase
MAPKK	MAPK kinase
MAPKKK	MAPK kinase kinase
MEK	MAPK kinase
MEM	modified Eagle's media
MCF-7	mammary epithelial carcinoma cell line
MDA-MB-231	mammary epithelial carcinoma cell line
MDR1	multidrug resistance protein 1
MDR3	multidrug resistance protein 3
MES-SA	human sarcoma cell line
MMLV	moloney murine leukemia virus
Mnase I	micrococcal nuclease
MRP1	multiple resistance-associated protein 1
MW	molecular weight
NADH	nicotinamide adenine dinucleotide
NF-κB	nuclear factor-κB
NFAT	nuclear factor of activated T cells
NF-Y	nuclear factor Y
NF-YA	A subunit of NF-Y
NF-YAL	NF-YA long isoform
NF-YAS	NF-YA short isoform
NF-YB	B subunit of NF-Y
NF-YC	C subunit of NF-Y
NIH3T3	murine fibroblast (Swiss 3T3) cell line
NLS	nuclear localization sequence

nt	nucleotide
N-terminal	amino terminal
ONPG	o-Nitrophenol β-D-Galacto-pyranoside
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PBSE	phosphate buffered saline EDTA
PCR	polymerase chain reaction
pGL3B	pGL3-Basic
pGL2C	pGL2-Control
P-gp	P-glycoprotein
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PMSF	phenyl methane sulfonyl fluoride
PVDF	polyvinyl difluoride
pSV-β-gal	pSV-β-galactosidase expression vector
RNase	ribonuclease
ROS	reactive oxygen species
RT	reverse transcriptase
RT-PCR	reverse transcription PCR
S/T	serine/threonine
SAPK	stress-activated protein kinase
SDS	sodium dodecyl sulphate
SEK	SAPK kinase
SH2	src homology domain 2
SH3	src homology domain 3
Sp1	specificity protein 1
Sp3	specificity protein 3
Stat	Signal Transducers and Activators of Transcription
SV40	Simian virus 40
T	thymine
$t_{½}$	half life

T25/T75	25/75 cm^2 tissue culture vented flasks
TAE	Tris acetate EDTA
TAFs	TBP-associated factors
TBE	Tris Boric acid EDTA
TBP	TATA-box binding protein
TBST	Tris buffered saline triton X-100
TEMED	N,N,N',N'-Tetramethylethylenediamine
T_m	melting temperature
topo IIα	topoisomerase IIα
topo IIβ	topoisomerase IIβ
TPA	12-*O*-tetradecanoylphorbol 13-acetate
Tris	Tris-(hydromethyl) aminomethane
tsp	transcription start point
TTP	thymidine triphosphate
UV	ultra violet light
VP-16^R	etoposide-resistant
VM-26^R	teniposide-resistant
YB-1	Y-box binding protein-1

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