Molecular Mechanisms of Drug Resistance in K562 Multidrug Resistant Leukaemic Cell Lines

Jodie Stephenson

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CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

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TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

ABBREVIATIONS

A	adenine or adenosine
ABC	ATP-binding cassette
AMP	adenosine monophosphate
ATCC	American type culture collection
ATP	adenosine triphosphate
5'-azadC	5'-azadeoxycytidine
bp	base pairs
BSA	bovine serum albumin
C	cytosine or cytidine
CAT	chloramphenicol acetyl transferase
cAMP	cyclic AMP
cDNA	complementary DNA
Ci	curie
$C0_2$	Carbon dioxide
CpG	CG dinucleotide
CRE	cAMP regulatort elements
CREB	cAMP-responsive element binding protein
CRS	cAMP response sequences
CTP	cytosine triphosphate
DEPC	diethylpyrocarbonate
DH_2O	deionised water
DMSO	dimethylsulphoxide
DNA	deoxribonucleic acid
DNase	deoxyribonuclease
dNTP	(unspecified) deoxyribonucleotide triphosphate
dsDNA	double stranded DNA
DTT	dithiothreitol
EDTA	ethylenediaminetetracetic acid
Egr	early growth response
EGTA	ethylene glycol-bis-(-aminoethyl ether) N, N, N', N'-tetracetic acid

EMSA	electrphoretic mobility shift assay
ET-743	Ecteinascidin-743
EtBr	ethidium bromide
5-FU	5-Fluorouracil
fmole	femptomole
g	grams
G	guanine or guanosine
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GSH	glutathione
GST	glutathione S-transferase
GTP	guanine triphosphate
HEPES	N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid
HSRE	heat shock response element
IC50	50% inhibitory concnentration
kb	kilobase
kDa	kilodalton
L	litre
MDR	multidrug resistance
MDR1	multidrug resistance gene 1
MDR2	multidrug resistance gene 2
mM	millimolar
$\mu J/cm^2$	microjoules per centimetre squared
μM	micromolar
ml	millilitre
mRNA	messenger RNA
MRP	multidrug resistance associated protein
MRP1	multidrug resistance associated protein gene 1
MTT	3-4,5-dimethylthiazole-2,5 diphenyl tetra bromide
MW	molecular weight
nm	nanometer
nM	nanomolar
NRE	negative response element

P/CAF	CREB binding protein associated factor
PCR	polymerase chain reaction
P-gp	p-glycoprotein
PKA	type 1 cAMP-dependant kinase
PKC	protein kinase C
pmole	picomole
PABP	poly(A)-binding protein
poly(A)	polyadenylated
RB	retinoblastoma
RNA	ribonucleic acid
RNase	ribonuclease
RT	room temperature
RT-PCR	reverse-transcriptase polymerase chain reaction
SDS	sodium dodecyl sulphate
SRE	stress response elements
SSC	sodium chloride sodium citrate
SSPE	Sodium chloride sodium phosphate + Ethylenediaminetetraacetic acid
SSRE	serum starvation response element
ssYB-1	single stranded YB-1
T	thymine or thymidine
TBE	Tris-borate buffer
TBP	TATA binding protein
TE	Tris EDTA
TPA	12-O-tetradecanoylphorbol-13-acetate
Tris	tris(hydroxymethyl) aminomethane
TSA	trichostatin A
TSS	transcription start site
tRNA	transfer RNA
TTP	thymidine triphosphate
U	unit
UV	ultraviolet

ABSTRACT

A major problem in chemotherapy is that many cancers are intrinsically drug resistant or later become resistant. Resistance to one drug is often accompanied by cross-resistance to many unrelated drugs and this is known as multidrug resistance (MDR). MDR is commonly associated with a 170kDa glycoprotein called P-glycoprotein (P-gp), which is thought to act as an ATP-dependant drug efflux pump and which is encoded for by the *MDR1* gene in humans. *MDR1* transcription can be initiated by various mechanisms such as demethylation of *MDR1* promoter sequences or translocation of the *MDR1* gene. In cells already expressing P-gp this expression can be increased by mechanisms such as amplification of the *MDR1* gene copy number or an increase in the rate of mRNA translation or stability. However the focus of many studies is on the regulation of *MDR1* transcription through the binding of transcription factors to specific sequences in the *MDR1* promoter region, in particular an inverted CCAAT element known as the Y-box and the –55GC box.

The current study investigated the mechanisms for MDR in a series of K562 derived MDR cell lines demonstrating varying levels of low level MDR. Levels of resistance of each of the K562 MDR cell lines were all confirmed by performing vinblastine and paclitaxel cytotoxicity assays on the cell lines. Northern hybridisation of total RNA isolated from K562 MDR cells indicated a positive correlation existed between the level of *MDR1* mRNA expressed in the MDR cells and the level of MDR displayed by the cells. Southern blot hybridisation of DNA from the cells with an *MDR1* probe indicated that increase of *MDR1* mRNA in the K562 MDR cells was not due to amplification of the *MDR1* gene. Bisulphite genomic sequencing of K562 and MDR cell lines revealed the *MDR1* promoter in both cell lines to be almost completely unmethylated apart from two distinct sites of methylation in K562 cells at two CpG sites downstream of the transcription start at +421 and +423bp respectively.

Treatment of the K562 cells with the histone deacetylase inhibitor Trichostatin A (TSA) increased the cells resistance to epirubicin, however no effect was seen upon TSA treatment of the K562 MDR cells. This suggests there is a difference in the chromatin structure in the two cell lines.

It was further demonstrated that resistance levels of K562 derived MDR cell lines declined with increasing time in drug free culture, but could be restimulated by short term drug exposure to P-gp substrate drugs, in some cases in as little as after 4 hours exposure, suggesting a transcriptional mechanism of *MDR1* upregulation is likely to be responsible for the induction. Consistent with this, mRNA stability studies indicated that the drug induction of K562 MDR cells that resulted in increased MDR and *MDR1* mRNA levels was not mediated by an increase in the stability of *MDR1* mRNA, as the rate of mRNA decay was the same in treated and untreated control cells. However, electrophoretic mobility shift assays (EMSA) of the two major transcription factors involved in *MDR1* regulation, the Y-box binding protein (identified as NF-Y) and the –55GC box binding protein indicated no difference in nuclear levels of these two proteins in untreated and drug induced cells. Thus the mechanism for up-regulation of *MDR1* activity in K562 MDR cell lines is most likely due to activation via alteration of other factors or via changes in chromatin structure regulating accessibility of transcription factors.