REGROWTH RESISTANCE IN PLATINUM-DRUG RESISTANT SMALL CELL LUNG CANCER CELLS

BRITTA STORDAL

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Certificate of Authorship/Originality

I hereby certify that the work in this thesis has not been previously submitted for a degree. I also certify that I wrote this thesis and any help received in my research work or in the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Britta Stordal
Bill Walsh Cancer Research Laboratories
Medical Oncology Department
Royal North Shore Hospital
University of Sydney.

Abstract

The H69CIS200 cisplatin-resistant and H69OX400 oxaliplatin-resistant cell lines developed as part of this study, are novel models of low-level platinum resistance. These resistant cell lines do not have common mechanisms of platinum resistance such as increased expression of glutathione or decreased platinum accumulation. Rather, these cell lines have alterations in their cell cycle allowing them to proliferate rapidly post drug treatment in a process known as 'regrowth resistance'. This alteration in cell cycle control has come at the expense of DNA repair capacity. The resistant cell lines show a decrease in nucleotide excision repair and homologous recombination repair, the reverse of what is normally associated with platinum resistance. The alterations in these DNA repair pathways help signal the G_1/S checkpoint to allow the cell cycle to progress despite the presence of DNA damage. The decrease in DNA repair capacity has also contributed to the development of chromosomal alterations in the resistant cell lines. Similarities in chromosomal change between the two platinum resistant cell lines have been attributed to inherent vulnerabilities in the parental H69 cells rather than part of the mechanism of resistance.

The H69CIS200 and H69OX400 resistant cells are cross-resistant to both cisplatin and oxaliplatin. This demonstrates that oxaliplatin does not have increased activity in low-level cisplatin-resistant cancer. Oxaliplatin resistance also developed more rapidly than cisplatin resistance suggesting that oxaliplatin may be less effective than cisplatin in the treatment of SCLC. The resistant cell lines have also become hypersensitive to taxol but show no alterations in the expression, polymerisation or morphology of tubulin. Rather, the PI3K/Akt/mTOR pathway is involved in both platinum resistance and taxol sensitivity as both are reversed with rapamycin treatment. mTOR is also phosphorylated in the resistant cell lines indicating that platinum resistance is associated with an increase in activity of this pathway.
The mechanism of regrowth resistance in the platinum-resistant H69CIS200 and H69OX400 cells is a combination of activation of PI3K/Akt/mTOR signalling and alterations in control of the G_1/S cell cycle checkpoint. However, more work remains to determine which factors in these pathways are governing this novel mechanism of platinum resistance.

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Table of Contents

List of Figures

List of Tables

Journal Publications

1) Stordal, B.K., Davey, M.W. and Davey, R.A. (2006). Oxaliplatin induces drug resistance more rapidly than cisplatin in H69 small cell lung cancer cells. Cancer Chemotherapy and Pharmacology. 58(2): 256-265.

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3) Stordal, B., Pavlakis N. and Davey, R. Oxaliplatin for the treatment of cisplatin-resistant cancer: a systematic review. Accepted for publication in Cancer Treatment Reviews 23.1.2007

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1) Stordal, B.K., Davey, M.W. and Davey, R.A. (2005). Oxaliplatin does not have activity against low-level cisplatin resistance in a lung cancer cell model. American Association for Cancer Research Annual Meeting, Anaheim California, USA. Poster – 1488.

2) Stordal, B.K, Peters, G.B., St. Heaps L. and Davey R.A. (2006). Oxaliplatin and cisplatin cause similar chromosomal changes in H69 SCLC cells: Linking changes in genotype to the resistant phenotype. American Association for Cancer Research Annual Meeting, Washington D.C, USA. Poster – 560.

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2) Stordal, B.K., Davey, M.W. and Davey, R.A. (2004). Comparative study on the development of cisplatin and oxaliplatin resistance in a lung cancer cell model. UTS/RNSH 21st Scientific Research Meeting. Sydney, Australia. Oral – O32.

3) Stordal, B.K, Peters, G., St. Heaps L., Davey M.W. and Davey R.A. (2005). Chromosome rearrangements and platinum drug resistance: Part of the resistance phenotype or "collateral damage"? UTS/RNSH 22nd Scientific Research Meeting. Sydney, Australia. Oral – O17.

4) Stordal B.K. and Davey R.A. (2006). Decreased rather than increased DNA repair proteins in platinum-resistant small cell lung cancer cell lines. UTS/RNSH 23rd Scientific Research Meeting. Sydney, Australia. Oral – O12.

List of Abbreviations

Abbreviation	Full Name
4E-BP	eIF-4E Binding Protein
Acc	Accumulation
ASK1	Apoptosis Signal-regulating Kinase 1
AMPS	Ammonium Persulfate
ATP	Adenosine Triphosphate
AP	Alkaline Phosphatase
bp	base pair
BAN	Biological Association Network
BER	Base Excision Repair
BCIP	5-bromo-4-chloro-3-indolyphosphate
BCNU	1,3-Bis(2-chloroethyl)-1-nitrosourea
BSA	Bovine Serum Albumin
BSO	Buthionine sulfoximine
Car	Carboplatin
cDNA	Complementary DNA
Cis	Cisplatin (*cis*-diamminedichloroplatinum (II))
DAPI	4',6-diamidino-2-phenylindole, dihydrochloride
dCK	deoxycytidine kinase
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
Dox	Doxorubicin
D-PBS	Dulbecco's Phosphate Buffered Saline
DTNB	5,5'-dithio-bis(2-Nitrobenzoic acid)
EDTA	Ethylene diamine tetra acetic acid
EGFR	Epidermal Growth Factor Receptor
EGTA	Ethyleneglycol-bis(β-amino-ethyl ether)N,N'-tetra acetic acid
EtBr	Ethidium bromide
ERCC1	Excision Repair Deficiency Complementing Group 1
FCS	Foetal Calf Serum

FISH	Fluorescent In Situ Hybridisation
FITC	Fluorescein isothiocyanate
Flav	Flavopiridol
FKBP	FK506-binding protein
γGCS	gamma-Glutamyl Cysteine Synthetase
γGCS-Cat	γGCS Catalytic subunit
γGCS-Reg	γGCS Regulatory subunit
GPX	Glutathione Peroxidase
GRX	Glutaredoxin
GSH	Glutathione
GSK3	Glycogen synthase kinase 3
GSR	Glutathione Reductase
GST	Glutathione-S-Transferase
γGT	gamma-Glutamyl Transpeptidase
Gy	Grey – Unit of measurement of radiation
H69	H69 Small Cell Lung Cancer Cell Line
H69CIS200	H69 Cisplatin Resistant Small Cell Lung Cancer Subline
H69OX400	H69 Oxaliplatin Resistant Small Cell Lung Cancer Subline
H69CIS200-S	Sensitive Revertant of H69CIS200
H69OX400-S	Sensitive Revertant of H69OX400
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRR	Homologous Recombination Repair
IC_{50}	50% Inhibitory Concentration
IKK	IκB kinase
JNK	c-Jun N-terminal Kinase
kb	Kilobase
kDa	Kilodaltons
LOH	Loss of Heterozygosity
LRP	Lung Resistance-related Protein (MVP)
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NPC	Nuclear Pore Complex
NSCLC	Non-Small Cell Lung Cancer
MAP kinase	Mitogen Activated Protein kinase

MAP4	Microtubule Associated Protein 4
MMR	Mismatch Repair
mRNA	messenger Ribonucleic Acid
MRP1	Multidrug Resistance-associated Protein 1 (ABCC1)
MRP2	Multidrug Resistance-associated Protein 2 (ABCC2)
MRP4	Multidrug Resistance-associated Protein 4 (ABCC4)
MOPS	3-[N-morpholino]propane-sulphonic acid (free acid)
MSH2	DNA mismatch repair protein 2
mTOR	mammalian Target Of Rapamycin
MTT	3-4,5-dimethylthiazol-2,5-diphenol tetrazolium bromide
MW	Molecular Weight
nd	not determined
NER	Nucleotide Excision Repair
NBT	Nitroblue tetrazolium
Ox	Oxaliplatin
p21	$p21^{WAF\text{-}1/Cip1}$
PAGE	Polyacrylamide Gel Electrophoresis
PBS	Phosphate Buffered Saline
PCR	Polymerise Chain Reaction
P-gp	P-glycoprotein (ABCB1)
PI3K	Phosphatidylinositol 3-kinase
PIP3	Phosphatidylinositol-3,4,5-trisphosphate
Pol	Polymerase
POLD1	DNA Polymerase Delta Catalytic Subunit
pRB	Retinoblastoma tumor suppressor gene
Pt	Platinum
RAD51B	DNA Repair Protein Rad51 Homolog 2
RAR	Retinoic Acid Receptor
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
RPMI	Culture Media (Roswell Park Memorial Institute)
RNase	Ribonuclease
S6K	ribosomal protein S6 Kinase
SSA	Sulphosalicyclic acid

SCLC	Small Cell Lung Cancer
SDS	Sodium Dodecyl Sulphate
SNP	Single Nucleotide Polymorphism
SSC	Sodium Chloride and Sodium Citrate Buffer
Tax	Taxol
TBE	Tris Borate Buffer with EDTA
TBS	Tris Buffered Saline
TE	Tris EDTA Buffer
TEMED	Tetramethylethylenediamine
Tm	Melting Temperature
TNF	Tumour Necrosis Factor
TopoI	Topoisomerase I
TopoII	Topoisomerase II
TRX	Thioredoxin
TrxR	Thioredoxin Reductase
Vin	Vindesine
XPA	Xeroderma Pigmentosum Complementing Protein