A SYSTEMATIC REVIEW OF GENES INVOLVED IN THE INVERSE RESISTANCE RELATIONSHIP BETWEEN CISPLATIN AND PACLITAXEL CHEMOTHERAPY : ROLE OF BRCA1

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ABSTRACT

A systematic review of cell models of acquired drug resistance not involving genetic manipulation showed that 80% of cell models had an inverse resistance relationship between cisplatin and paclitaxel[1]. Here we systematically review genetically modified cell lines in which the inverse cisplatin/paclitaxel resistance phenotype has resulted. This will form a short list of genes which may play a role in the mechanism of the inverse resistance relationship as well as potential markers for monitoring the development of resistance in the clinical treatment of cancer. The literature search revealed 91 genetically modified cell lines which report toxicity or viability/apoptosis data for cisplatin and paclitaxel relative to their parental cell lines. This resulted in 26 genes being associated with the inverse cisplatin/paclitaxel phenotype. The gene with the highest number of genetically modified cell lines associated with the inverse resistance relationship was BRCA1 and this gene is discussed in detail with reference to chemotherapy response in cell lines and in the clinical treatment of breast, ovarian and lung cancer. Other genes associated with the inverse resistance phenotype included dihydrodiol dehydrogenase (DDH) and P-glycoprotein. Genes which caused cross resistance or cross sensitivity between cisplatin and paclitaxel were also examined, the majority of these genes were apoptosis associated genes which may be useful for predicting cross resistance. We propose that BRCA1 should be the first of a panel of cellular markers to predict the inverse cisplatin/paclitaxel resistance phenotype.

INTRODUCTION

The chemotherapeutic drugs cisplatin and paclitaxel are used in the treatment of many solid tumours. Cisplatin binds to the DNA strand, hindering both DNA replication and RNA translation and eventually triggering apoptosis. Paclitaxel causes cytotoxicity by binding to and stabilising polymerised microtubules. Initial responsiveness to cisplatin therapy is high, however the majority of patients ultimately relapse with resistant disease. Mechanisms of cisplatin resistance characterised in resistant cell models include; decreased cellular accumulation of drug, increased levels of glutathione, increased levels of DNA repair and increased anti-apoptotic activity [2]. Similarly, many patients will relapse with disease resistant to paclitaxel therapy. Paclitaxel resistance can be mediated by P-glycoprotein export decreasing the cellular accumulation [3]. Other mechanisms of paclitaxel resistance include altered expression or post-translational modification of β-tubulin, the target of paclitaxel, or other microtubule regulatory proteins. Any alteration in microtubule dynamics, paclitaxel binding sites or signalling pathways up or downstream of microtubule polymerisation can potentially mediate paclitaxel resistance [4].

Due to their differing mechanisms of action cisplatin and paclitaxel are often combined in cancer therapy. However, work in cell lines suggests that alternating between the two classes of drugs may be beneficial. In a recent systematic review article we examined the cross resistance relationship between cisplatin and paclitaxel in cell models of acquired drug resistance [1]. The vast majority of cell models had an inverse resistance relationship between cisplatin and paclitaxel. When cisplatin resistance occurs cell lines are likely to have no change in resistance to paclitaxel and some cells even become hypersensitive to paclitaxel. The reverse is also true of paclitaxel-resistant cell lines, which either show no change in resistance to cisplatin or have become hypersensitive to cisplatin. This inverse resistance relationship is also present between other platinum drugs such as carboplatin and the newer taxane docetaxel. The inverse resistance relationship was present in drug-resistant cell models developed with both platinums or taxanes as well as those developed with other classes of chemotherapeutics such as anthracyclines, topoisomerase inhibitors, anti-metabolites and even radiation. This suggested the involvement of cellular

pathways that respond to a broad variety of drugs to produce either platinum resistance accompanied by taxane sensitivity or taxane resistance and platinum sensitivity.

The genetic modification of cell lines by over or under expressing genes can provide insight into which genes are involved in resistance to a given chemotherapeutic. These studies will often amplify or reduce the expression of a gene to a greater extent to what would be observed in the development of drug resistance in cancer patients but nevertheless provide insight into which pathways can mediate resistance. Transfection studies have linked the overexpression of copper efflux transporters ATP7A [5] and ATP7B [6] with cisplatin resistance. Decreasing the expression of DNA repair gene ERCC1 by siRNA [7] or glutathione transferase GSTP1[8] by antisense has been shown to mediate sensitivity to cisplatin. Similarly, the overexpression of ABC transporter P-glycoprotein has been shown to mediate paclitaxel resistance [9].

We undertook this systematic review to determine which genetic modifications in cell lines have created the inverse cisplatin/paclitaxel resistance phenotype. This will form a short list of genes which may play a role in the mechanism of the inverse resistance relationship as well as potential markers for monitoring the development of resistance in the clinical treatment of cancer.

METHODS

Medline was searched for cell lines which had been genetically modified and reported toxicity data for both cisplatin and paclitaxel. The following terms were used as keywords: 'cisplatin', 'taxol', 'paclitaxel', 'cross resistance', 'cross resistant', 'resistant', 'resistance', 'toxicity', 'sensitive', 'sensitivity', 'IC50', 'transfection', 'overexpression', 'RNAi', 'antisense', 'siRNA', 'ribozyme', 'knockout', 'knockdown' and 'cell line'. Review articles and articles not published in English were excluded. Conference presentations and abstracts were not included. The literature searches were last updated in October 2008.

Resistant cell models developed by selection with chemotherapy which have then been treated by transfection or antisense to reverse resistance were not included in the

systematic review process, but were used when appropriate to strengthen the case for identified genes being involved in the inverse resistance phenotype. Cell lines which had multiple genetic modifications were excluded.

RESULTS AND DISCUSSION

Types of Genetic Modification

There are many experimental techniques to over or under express genes of interest to produce genetically modified cell lines for the study of genes and pathways involved in drug resistance. Transfection is the process of introducing nucleic acids into cells by non-viral methods. Most commonly plasmid DNA containing the sequence of a gene of interest is introduced into mammalian cells by methods such as electroporation or liposome-mediated transfer. This results in the gene of interest being overexpressed in the host cell. This is most commonly transient overexpression but stable transfectants can be selected where the plasmid DNA has been integrated into the chromosome of the cell [10]. Adenovirus vectors can also be used to overexpress a gene of interest [11]. Transfection and adenoviruses can also be used to inhibit gene expression if they are used to introduce antisense oligonucleotides or generate small interfering RNAs (siRNA), described below.

Antisense and RNA interference (RNAi) inhibition of gene expression are two methods which mediate post-transcriptional gene silencing based on complementary base pairing to the mRNA to be inhibited. Antisense uses single-stranded oligonucleotides of 13-15 bases which are complementary to a specific gene. The binding of the oligonucleotide inhibits the translation of the gene into a protein, via hybridizing to corresponding mRNA [12]. A ribozyme is an RNA molecule that can catalyse a chemical reaction. A Ribozyme catalytic centre is incorporated into antisense RNA and specifically cleaves and destroys the target RNA. This provides an advantage over standard antisense technology which only inactivates the target RNA without degrading it [13]. RNAi uses double-stranded RNAs (dsRNA) homologous to the gene being suppressed. Long dsRNAs are processed within the cell by dicer, a cellular ribonuclease III, to generate duplexes of about 21 nucleotides with 3'-

overhangs known as small interfering RNAs (siRNAs) which mediate mRNA degradation [14].

A gene can be knocked out from an animal in order to investigate the absence of a specific gene product. Gene knockout technology arose from the combination of two techniques: the culture of multipotent embryonic stem cells from mouse embryos and the induction of mutations into mammalian cells by homologous recombination. The embryonic stem cells could be isolated, manipulated in culture and then reintroduced into a wild type embryo. Knocking-in uses the same technique of homologous recombination as the knock-out strategy, but the targeting vector is designed to enhance the function of the gene of interest rather than disrupt it [15]. Fibroblasts from knockout or knock-in mice are often studied in culture for changes in resistance to chemotherapy agents.

Defining Resistance

When genetically modified cell lines are developed in the laboratory their levels of resistance can be compared to their parental unmodified cells using a cell viability assay such as the MTT or clonogenic assay. The cisplatin and paclitaxel sensitivity of these paired cell lines is usually determined by exposing them to a range of drug concentrations and then assessing cell viability. The IC_{50} (drug concentration causing 50% growth inhibition) for these paired cell lines can be used to determine the increase or decrease in resistance, known as fold resistance, by the following equation:-

$$\text{Fold Resistance} = \frac{IC_{50} \text{ of Genetically Modified Cell Line}}{IC_{50} \text{ of Unmodified Parental Cell Line}}$$

The definition of cross resistance is a matter of debate in the literature. Some studies consider two drugs cross-resistant only if a similar level of resistance is observed. Studies which have developed cell lines from patients before and after chemotherapy have found that drug resistance in the clinic typically produces resistance of 2 to 3-fold [16,17]. For the purposes of this review we have defined cross resistance between cisplatin and paclitaxel as greater than or equal to 2-fold resistance to both drugs. This

definition is therefore based on what would be clinically observed as cross resistance. We have defined cross sensitivity as less than or equal to 0.8-fold resistance to both drugs. (Figure 1, indicated in black)

Alternatively, many studies will not report an IC_{50} for their genetically modified cell lines. Rather, resistance or sensitivity will be defined by exposing the cell lines to a single dose of drug and then assaying for cell viability or level of apoptosis. We have reported the conclusions of the authors of these studies. Resistance was usually defined as a significant increase in viability or decrease in apoptosis after exposure to chemotherapy.

The literature search revealed 91 genetically modified cell lines which report toxicity or viability/apoptosis data for cisplatin and paclitaxel relative to their parental cell lines. There are three categories of the inverse cisplatin/paclitaxel resistance phenotype. Non-cross resistance is where the genetic modification has induced resistance to one drug with no change to the other compound. Hypersensitivity is resistance to one drug which has induced sensitivity to the other compound (Figure 1). Both these categories are likely to occur in the clinical treatment of cancer, where resistance develops to one compound but sensitivity is present to the other. The third category is non-cross sensitivity, where the genetic modification has produced sensitivity to one agent and not altered the toxicity of the other. This category is unlikely to occur in the clinic but genetic modifications which produce this category will aid in our understanding of the inverse resistance phenotype. The areas in Figure 1 shaded in grey are the categories of genetic modification that will aid our understanding of cisplatin resistance and paclitaxel sensitivity. The areas with grey stripes are the opposite phenotype, paclitaxel resistance and cisplatin sensitivity.

Genetic Modifications Which Induce the Inverse Cisplatin/Paclitaxel Phenotype

Tables 1 and 2 summarise the genetically modified cell lines which correlate with the inverse cisplatin/paclitaxel resistance phenotype. Table 1 covers cisplatin resistance and paclitaxel sensitivity and Table 2 covers paclitaxel resistance and cisplatin sensitivity. A total of 26 genes may therefore be associated with the inverse resistance phenotype.

Table One – Genetic Modifications Producing Cisplatin Resistance and Paclitaxel Sensitivity

Cell Line	Cancer	Modification	Method	Cisplatin	Paclitaxel	Reference	Mechanism of Resistance
Cisplatin Resistant - Paclitaxel Sensitive							
HCC1937	Breast	↑BRCA1	Transfection	Resistant	Sensitive	[18]	↓Apoptosis in response to cisplatin, ↑Apoptosis in response to paclitaxel
HCC1937	Breast	↑BRCA1	Transfection	20.5	0.001	[19]	
2008	Ovarian	↑DDH	Transfection	7.7	0.72	[20]	Increase in cellular detoxification by dihydrodiol dehydrogenase (DDH) -cisplatin not direct substrate
A431	Cervical	↑DDH	Transfection	2.2	0.78		
Calu	Lung Adenocarcinoma	↑DDH	Transfection	6.3	0.59		
Cisplatin Resistant - Paclitaxel No Change							
A549	NSCLC	↑AKT1	Transfection	2.5	1.3	[21]	Possible mechanism - Decrease in apoptosis in response to cisplatin
MiaPaCa2	Pancreatic	↑ASNS	Transfection	Resistant	No Change	[22]	Increased asparagine synthetase leads to ↓Apoptosis in response to cisplatin under glucose deprived conditions
SKOV	Ovarian	↑DDH	Transfection	3	1.02	[20]	Increase in cellular detoxification by dihydrodiol dehydrogenase (DDH) -cisplatin not direct substrate
A2780	Ovarian	↑DDH	Transfection	4	0.93		
Tera	Germ Cell	↑DDH	Transfection	46	0.85		
CH1	Ovarian	↑erbB2	Transfection	2.1	1.42	[23]	Mechanism not investigated
MCF-7	Breast	↑H-Ras	Transfection	9.5	1.1	[24]	Decrease in DNA fragmentation induced by cisplatin
MCF-7	Breast	↑H-Ras	Transfection	3.8	1		
H460	NSCLC	↑MRIT/cFLIP	Transfection	Resistant	No Change	[25]	Decreased Apoptosis in response to cisplatin, by blocking the activation of caspase-8
A2780	Ovarian	↑mutant p53	Transfection	2.5	1.1	[26]	Mechanism not investigated
SKBR-3	Breast	↓PKCε	Antisense	2.7	1.1	[27]	Decrease in DNA fragmentation induced by cisplatin
Cisplatin No Change – Paclitaxel Sensitive							
A549	NSCLC	↓1kBa	Adenovirus	1.625	0.183	[28]	1kBa Inhibits NFkB, Caspase-3 activity increased in response to paclitaxel Apoptosis
MBR62	Breast	↑BRCA1	Transfection	No change	Sensitive	[29]	G2M arrest in response to paclitaxel, induction of GADD45
U-20S	Osteogenic Sarcoma	↑E2F-1	Transfection	No change	Sensitive	[30]	E2F-1(transcription factor) cyclin B1 levels and cdc2 kinase activity becoming sensitive to paclitaxel
A549	Lung	↑HERG	Transfection	0.875	0.039	[31]	Potassium Channel, mechanism of paclitaxel sensitivity unknown
SH-EP	Neuroblastoma	↑MYCN	Transfection	0.94	0.51	[32]	Mechanism of paclitaxel sensitivity not studied

Table Two – Genetic Modifications Producing Paclitaxel Resistance and Cisplatin Sensitivity

Cell Line	Cancer	Modification	Method	Cisplatin	Paclitaxel	Reference	Mechanism of Resistance
Paclitaxel Resistant - Cisplatin Sensitive							
HBL100	Breast	↓BRCA1	Ribozyme	Sensitive	Resistant	[33]	↓BRCA1 leads to transcriptional modifications of the JNK pathway ↑JNK1 ↓JNK2
MCF-10A	Mammary Epithelial	↑c-erbB2	Transfection	0.625	3.5	[34]	c-erbB2 is member of the EGFR family, mechanism of resistance/sensitivity unknown but likely due to alteration of growth or apoptotic pathways
OAW42	Ovarian	↑Survivin	Transfection	0.8	6.75	[35]	Increased expression of anti-apoptotic survivin ↓Apoptosis
Paclitaxel Resistant – Cisplatin No Change							
OVCAR3	Ovarian	↑Bcl-Xl	Transfection	No change	Resistant	[36]	Increased expression of anti-apoptotic Bcl-Xl ↓Apoptosis
SKOV3	Ovarian	↓HER-2	Ribozyme	1	79	[37]	Cells with decreased HER-2 accumulate in S-phase (SKOV3 cells normally very high HER-2)
OVCAR8	Ovarian	↑MAGE2	Transfection	1	4	[38]	Increased Proliferation, No change in P-glycoprotein
OVCAR8	Ovarian	↑MAGE6	Transfection	1	4		
HeLa	Ovarian	↑MRP1	Transfection	0.9	2	[39]	MRP1 is a poor transporter of paclitaxel - low level resistance
U-20S	Osteogenic Sarcoma	↑PGK1	Transfection	1	30	[40]	Increase in Phosphoglycerate Kinase 1 possibly mediating increased glycolysis, no change in P-gp
MCF-7	Breast	↑P-gp	Transfection	1.2	36	[9]	Increased P-glycoprotein-mediated efflux of Paclitaxel
IGROV-1	Ovarian	↑Survivin	Transfection	1.25	6.6	[35]	Increased expression of anti-apoptotic survivin ↓Apoptosis
Paclitaxel No Change – Cisplatin Sensitive							
OSE	Ovarian (Mouse)	↓BRCA1	Knockout mouse	Sensitive	No Change	[41]	Increase in chromosome breaks and other abberations due to cisplatin
Lymphocytes	Lymphocytes (Human)	↓BRCA1	BRCA1 mutation	Sensitive	No Change	[42]	Patients with BRCA mutation compared to healthy controls, mechanism not investigated
H460	NSCLC	↑FHIT	Adenovirus	0.57	1	[43]	Fragile Histidine Triad (FHIT), Apoptosis
M7609	Colon	↓GSTP1	Antisense	0.41	1.33	[8]	Decrease in detoxification of cisplatin
M7609	Colon	↓GSTP1	Antisense	0.44	0.85		
HCT-116	Colon	↓p21	Knockout	0.34	0.98	[44]	Reduced DNA repair of damage due to cisplatin
HCT-116	Colon	↓p53	E6 virus	0.23	0.98		
H157	Lung	↑p53wt	Adenovirus	0.38	1.11	[45]	Mechanism of Cisplatin sensitivity not investigated
H1299	Lung	↑p53wt	Adenovirus	0.37	1.17		

HeLa	Ovarian	↑p53wt	Adenovirus	0.68	0.93	[46]	Increase in DNA fragmentation induced by cisplatin
HFF	Human Fibroblasts	↓RB	E7 virus	0.53	0.93	[47]	Mechanism of Cisplatin sensitivity not investigated
MEF	Mouse Fibroblasts	↓Xrcc2	Knockout mouse	Sensitive	No Change	[48]	Decrease in Homologous Recombination repair of DNA double strand breaks induced by cisplatin

From the literature review the gene which was correlated most highly with the inverse cisplatin/paclitaxel resistance phenotype was BRCA1 in six different studies [18,19,29,33,41,42]. BRCA1 has been intensively investigated in many studies due to its role in familial breast and ovarian cancer, but has more recently been studied as a chemotherapy response marker in cell lines and in the clinic. When BRCA1 expression is increased this leads to cisplatin resistance and paclitaxel sensitivity [18,19]. Conversely, when BRCA1 expression is decreased this leads to paclitaxel resistance and cisplatin sensitivity [33]. BRCA1 is the only gene identified which appears on both Tables 1 and 2, showing that both increases and decreases in expression correlate with the inverse resistance phenotype. BRCA1's potential involvement with the inverse resistance mechanism will be discussed in detail later in this review article.

Increasing the expression of anti-apoptotic survivin has been shown to cause paclitaxel resistance in several studies [49] and no change or sensitivity to cisplatin in two ovarian cancer cell lines [35]. However, in studies which have only examined the toxicity of cisplatin and not paclitaxel, decreasing the expression of survivin caused cisplatin sensitivity and not resistance suggesting that survivin may not be involved in the inverse cisplatin/paclitaxel resistance phenotype [50,51].

Increased expression of dihydrodiol dehydrogenase (DDH) has caused cisplatin resistance and sensitivity to paclitaxel in a panel of cell lines including ovarian, cervical and lung cancers [20]. Increased DDH was thought to mediate cisplatin resistance by increasing the detoxification capability of the cell, although cisplatin is not a direct substrate of DDH. Increased expression of DDH has also been correlated with cisplatin resistance in ovarian cancer patients [52]. Decreasing the expression of DDH has been investigated, but the toxicity of cisplatin and paclitaxel were not examined [53]. However, the cells were rendered more sensitive to DNA binding drug bleomycin [53]. The role of DDH in the inverse resistance phenotype requires further study. Examining the toxicity of cisplatin and paclitaxel in cells with decreased DDH expression would be an important starting point.

Paclitaxel resistance can be mediated by P-glycoprotein export decreasing the cellular accumulation [3]. Surprisingly, the literature search only revealed one cellular model

of increased paclitaxel resistance mediated by transfection of P-glycoprotein which also reported cisplatin toxicity data [9]. This is most likely due to two factors: 1) Paclitaxel resistance mediated by P-glycoprotein is relatively easy to induce with paclitaxel treatment. 2) When P-glycoprotein has been transfected into cells cisplatin resistance may not have been examined as cisplatin is not a substrate of P-glycoprotein. However, we know from work in resistant cell models developed with paclitaxel treatment that P-glycoprotein is associated with the inverse cisplatin/paclitaxel resistance phenotype. Resistant cell models developed using paclitaxel in nasal septum [54], osteosarcoma [55] and ovarian cancer cells [3,56] have shown levels of paclitaxel resistance ranging from 8 to 1500-fold mediated by P-glycoprotein. There was no cross resistance to cisplatin in these cell models, many of which had become hypersensitive to cisplatin.

There are several limitations to the identification of genes involved in the inverse cisplatin/paclitaxel resistance mechanism by searching the literature. Studies which investigate resistance to cisplatin after genetic manipulations will often only examine cross resistance to other platinum agents, heavy metals or other DNA targeting chemotherapy [6,7]. Similarly, studies investigating resistance to paclitaxel may only examine cross resistance to other microtubule targeting agents and or P-glycoprotein substrates and not cisplatin [57]. The other major limitation of this method is that it only examines genes which have already been identified, and the more popular the gene in terms of number of laboratories investigating it, the more likely it is to be examined in regards to both cisplatin and paclitaxel toxicities. Microarray-based studies designed to investigate the inverse resistance phenotype will no doubt reveal many other genes involved in the mechanism which have not been previously investigated.

Cisplatin/Paclitaxel Cross Resistance or Cross Sensitivity

The genetic modifications which have induced cisplatin/paclitaxel cross resistance or cross sensitivity are itemised in Table 3. These genes are unlikely to be involved in

Table Three – Genetic Modifications Producing Cisplatin/Paclitaxel Cross Resistance or Cross Sensitivity

Cell Line	Cancer	Modification	Method	Cisplatin	Paclitaxel	Reference	Mechanism of Resistance
Cross Resistance							
CHO	Ovarian (Hamster)	↑ATP7A	Transfection	2.4	14.6	[5]	Increased in cisplatin efflux mediated by copper transporter. Paclitaxel resistance unexplained, no efflux or presence of P-gp
CHO	Ovarian (Hamster)	↑ATP7A	Transfection	2.5	324.95		
Me32a	Fibroblast	↑ATP7A	Transfection	2.4	93.39		
A2780	Ovarian	↑Aurora-A	Transfection	Resistant	Resistant	[58]	Aurora-A induces survival by activating Akt growth signalling
C33A	Cervical	↑BAG-1	Transfection	Resistant	Resistant	[59]	Increased expression of anti-apoptotic BAG-1 ↓Apoptosis
A2780	Ovarian	↑Bcl-Xl	Transfection	Resistant	Resistant	[60]	Increased expression of anti-apoptotic Bcl-Xl ↓Apoptosis
HeLa	Cervical	↓COX-2	siRNA	Resistant	Resistant	[61]	↓Apoptosis in response to both cisplatin and paclitaxel
H1299	NSCLC	↓E2F1	RNAi	Resistant	Resistant	[62]	↓Apoptosis in response to both cisplatin and paclitaxel
MEFS	Mice Fibroblasts	↓E2F1	Knockout	Resistant	Resistant		
SKOV3	Ovarian	↓HtrA1	Antisense	Resistant	Resistant	[63]	Decreased expression of pro-apoptotic HtrA1 ↓Apoptosis
EJ	Bladder	↑p16	Adenovirus	Resistant	Resistant	[64]	Resistance mediated by growth arrest by replacement of functional p16 gene in p16 negative EJ cells
HEC-1	Endometrial	↑PXR	Transfection	Resistant	Resistant	[65]	↓Apoptosis in response to both cisplatin and paclitaxel
Cross Sensitivity							
A2780	Ovarian	↑Bax	Adenovirus	0.18	0.48	[66]	Increased expression of pro-apoptotic Bax ↑Apoptosis
OVCAR-3	Ovarian	↑Bax	Adenovirus	0.17	0.17		
MNK45	Gastric	↑Bax	Transfection	0.2	0.36	[67]	
MNK45	Gastric	↓Bcl-2	Antisense	0.34	0.28	[68]	Decreased expression of anti-apoptotic Bcl-2 ↑Apoptosis
8305C	Thyroid	↓Bcl-2	Antisense	Sensitive	Sensitive	[69]	
HUH6	Liver	↓Bcl-2	siRNA	Sensitive	Sensitive	[70]	
CaOV3	Ovarian	↓Bcl-XL	Antisense	Sensitive	Sensitive	[36]	Decreased expression of anti-apoptotic Bcl-Xl ↑Apoptosis
OVCAR3	Ovarian	↓Bcl-XL	Antisense	Sensitive	Sensitive		
SKOV3	Ovarian	↓Bcl-XL	Antisense	Sensitive	Sensitive		
H460	NSCLC	↓βIII Tubulin	siRNA	Sensitive	Sensitive	[71]	↑Apoptosis in response to both cisplatin and paclitaxel, altered microtubules and cell cycle in response to paclitaxel
Calu-6	NSCLC	↓βIII Tubulin	siRNA	Sensitive	Sensitive		
SKOV3ip1	Ovarian	↑E1A	Adenovirus	Sensitive	Sensitive	[72]	↑Apoptosis, Increased DNA fragmentation in response to paclitaxel
MEFS	Mice Fibroblasts	↓E2F4	Knockout	Sensitive	Sensitive	[62]	↑Apoptosis in response to both cisplatin and pacliaxel

293	Embryonic Kidney	↓E2F4	siRNA	Sensitive	Sensitive		
A549	NSCLC	↓EGFR	Antisense	0.15	0.15	[73]	Inhibition of growth factor Epidermal Growth Factor Receptor (EGFR)
SPC-A1	Lung Adenocarcinoma	↓EGFR	Antisense	0.17	0.16		
MNK45	Gastric	↑Gadd153	Transfection	0.18	0.69	[74]	Possible pro-apoptotic gene
MCF-10A	Mammary Epithelial	↑Ha-Ras	Transfection	0.625	0.4375	[34]	Oncogene, mechanism of sensitivity not investigated
OV167	Ovarian	↑HtrA1	Adenovirus	Sensitive	Sensitive	[63]	Serine protease HtrA1, induces cell death and activates caspase3/7 Apoptosis
CNE1	Nasopharygeal	↓Id-1	siRNA	Sensitive	Sensitive	[75]	Decreased expression of anti-apoptotic Id-1 ↑Apoptosis
DU145	Prostate	↓IGF1R	Antisense	Sensitive	Sensitive	[76]	Inhibition of growth factor type 1 insulin-like growth factor receptor
OV167	Ovarian	↑MCJ	Transfection	0.5	0.28	[77]	MCJ member of DNAJ family, more sensitive to paclitaxel induced apoptosis
SH-EP	Neuroblastoma	↑MYCN	Transfection	Sensitive	Sensitive	[78]	↑Apoptosis in response to both cisplatin and paclitaxel
HFF	Human Fibroblasts	↓p53	E6 virus	0.13	0.12	[47]	Cell cycle alteration in response to cisplatin treatment
PC-3	Prostate	↓PDPK Fa	Antisense	Sensitive	Sensitive	[79]	Proline-directed protein kinase FA, mechanism of sensitivity unknown
HEC-1	Endometrial	↓PXR	siRNA	Sensitive	Sensitive	[65]	↑Apoptosis in response to both cisplatin and paclitaxel
KYSE-150	Esophageal	↑PUMA	Adenovirus	Sensitive	Sensitive	[80]	Increased expression of pro-apoptotic p53 upregulated modulator of apoptosis (PUMA) Apoptosis
KYSE-140	Esophageal	↑PUMA	Adenovirus	Sensitive	Sensitive		
KYSE-510	Esophageal	↑PUMA	Adenovirus	Sensitive	Sensitive		
YES-2	Esophageal	↑PUMA	Adenovirus	Sensitive	Sensitive		
U87-MG	Glioma	↓hTR	Antisense	Sensitive	Sensitive	[81]	Decreased telomerase activity
U373-MG	Glioma	↓hTR	Antisense	Sensitive	Sensitive		
H460	NSCLC	↑TRAIL	Adenovirus	0.1	0.006	[82]	↑Apoptosis in response to both cisplatin and paclitaxel - TRAIL (TNF-related apoptosis inducing ligand)
T24	Bladder	↓TXAS	siRNA	0.55	0.27	[83]	Unknown
TCC-SUP	Bladder	↓TXAS	siRNA	0.72	0.48	[83]	Unknown

the inverse cisplatin/paclitaxel resistance phenotype but may be useful in predicting cross resistance between cisplatin and paclitaxel. It is interesting to note which genes cause generalised resistance or sensitivity to two different classes of chemotherapy. Some of these include well characterised apoptosis genes, decreased expression of anti-apoptotic Bcl-2 [68,69] and Bcl-Xl [36] and increased expression of pro-apoptotic Bax [66,67] all cause sensitivity to both cisplatin and paclitaxel. Similarly increased expression of Bcl-Xl by transfection produced cross resistance to cisplatin and paclitaxel [60].

The overexpression of ATP7A by transfection produced cross resistance between cisplatin and paclitaxel which was unexpected [5]. Low-level resistance to cisplatin was mediated by ATP7As role as an efflux protein for platinum [84]. The resistance to paclitaxel was higher and ranged from 14 to 325 – fold resistance. The mechanism of this resistance remains unexplained, but was not mediated by increased expression of P-glycoprotein or increased efflux of paclitaxel from the cell by any other transporter.

Role of BRCA1 in the Inverse Cisplatin/Paclitaxel Resistance Phenotype

Our systematic review of the literature found that when BRCA1 expression is increased cisplatin resistance and paclitaxel sensitivity occurs [18,19] and, conversely, when BRCA1 expression is decreased this leads to paclitaxel resistance and cisplatin sensitivity occurs [33]. BRCA1's role in the mechanism of cisplatin or paclitaxel resistance was the subject of a recent review article by Mullen et al 2006 in Biochimica and Biophysica Acta [85] so here we will only cover the main pathways influenced by BRCA1 (Figure 2), DNA repair and apoptosis, rather than a complete review of all the genes BRCA1 has been shown to regulate.

In cisplatin-resistant MCF-7 breast cancer cells BRCA1 up-regulation is associated with DNA repair mediated resistance to cisplatin [86]. Antisense inhibition of BRCA1 in this same cisplatin-resistant model resulted in an increased sensitivity to cisplatin, a decreased proficiency of DNA repair and an enhanced rate of apoptosis. BRCA1 has been found to be required for the subnuclear assembly of homologous recombination repair protein RAD51 into foci at the site of DNA double-strand breaks due to cisplatin [87,88].

BRCA1 deficiency confers resistance to paclitaxel and has been associated with a defective apoptotic response in BRCA1-deficient cells, suggesting that BRCA1 could regulate apoptotic pathways [19] (Figure 2). BRCA1 deficiency has also been shown to mediate paclitaxel resistance through premature inactivation of the spindle checkpoint at the metaphase anaphase transition [89] and through alterations of the JNK signalling pathway [33].

There may be an overall inverse resistance relationship between DNA targeting chemotherapy and microtubule targeting chemotherapy in which BRCA1 participates. Increased BRCA1 expression by transfection in HCC1937 breast cancer cells leads to increased resistance to DNA damaging agents etoposide and bleomycin as well as cisplatin [19]. Sensitivity to vinorelbine was also associated with increased BRCA1 expression along with paclitaxel [19].

Clinical Role for BRCA1 in Predicting Treatment Outcomes

BRCA1 is an important genetic factor in hereditary breast and ovarian cancer and there is increasing evidence of an important role for BRCA1 in the sporadic forms of both cancer types [85]. Therefore we sought to determine if BRCA1 mutations or alterations in BRCA1 mRNA and protein expression influence the response to cisplatin or paclitaxel-based chemotherapy.

Cisplatin combination chemotherapy is the cornerstone of treatment of ovarian carcinomas. Initial platinum responsiveness in ovarian cancer is high, but up to 80% of patients will eventually relapse and become cisplatin resistant [90]. Ovarian cancer patients with BRCA1 mutations have a higher 5-year survival rate (78.6%) compared to sporadic ovarian cancer (30.3%) [91]. All patients in this study were treated with at least 2 courses of cisplatin-based chemotherapy after surgery. This correlates with the *in vitro* data, as a decrease in BRCA1 should promote sensitivity to cisplatin. However, when the response rate of the first-line cisplatin chemotherapy regimen is examined the total of complete and partial responses is the same between patients with a BRCA1 mutation and that of sporadic ovarian cancer. The ratio of complete to partial responses was slightly higher in the BRCA1 mutation cohort but due to the

small number of patients (7) it is unclear if this difference caused the large difference in survival 5 years later. Other studies have also found increased survival of BRCA1 ovarian cancer patients over a five year period who have been treated with cisplatin-based therapy [92,93]. However, it is difficult to determine if the choice of cisplatin-based chemotherapy at first line is causing the dramatic difference in outcome 5 years later as the salvage chemotherapy treatments given to the BRCA1 patients are often not recorded. Complicating this further is the fact that paclitaxel is often given as salvage therapy in ovarian cancer or combined with cisplatin in first-line therapy [1] and BRCA1 mutation patients should be less responsive to paclitaxel therapy.

BRCA1 mRNA and protein expression levels have also been examined in tumours and compared to response to chemotherapy and 5-year survival. Low BRCA1 mRNA levels correlated with increased response to cisplatin/gemcitabine chemotherapy and increased 5-year survival in a group of 55 non-small cell lung cancer patients [94]. This again correlates with the in vitro data, as a decrease in BRCA1 should promote sensitivity to cisplatin. Low BRCA1 protein expression has also been shown to correlate with a shorter time to progression after taxane-based chemotherapy in breast cancer [95]. This also correlates with the in vitro data, as a decrease in BRCA1 should promote resistance to taxanes such as paclitaxel. However, there was no decrease in response to taxane-based chemotherapy in these patients, just a shorter time to progression [95]. Decreased levels of BRCA1 protein have also been shown to correlate with improved 5-year survival in breast cancer to other treatment regimens such as surgery and adjuvant radiotherapy [96] and combination treatment with cyclophosphamide, epirubicin and 5-fluorouracil [97].

CONCLUSIONS

BRCA1 is not the only gene responsible for the inverse cisplatin/paclitaxel resistance phenotype. BRCA1 may be over represented in the literature because of the interest in this gene in hereditary cancers. However, due to the large body of literature on BRCA1 we propose that it could be the first of a panel of cellular markers to predict the inverse cisplatin/paclitaxel resistance phenotype. BRCA1 mRNA or protein expression levels need to be further examined in tumour banks and correlated with both response to first and second line chemotherapy as well as time to progression and

5-year survival in order to fully understand the role of this protein in the inverse cisplatin/paclitaxel resistance phenotype.

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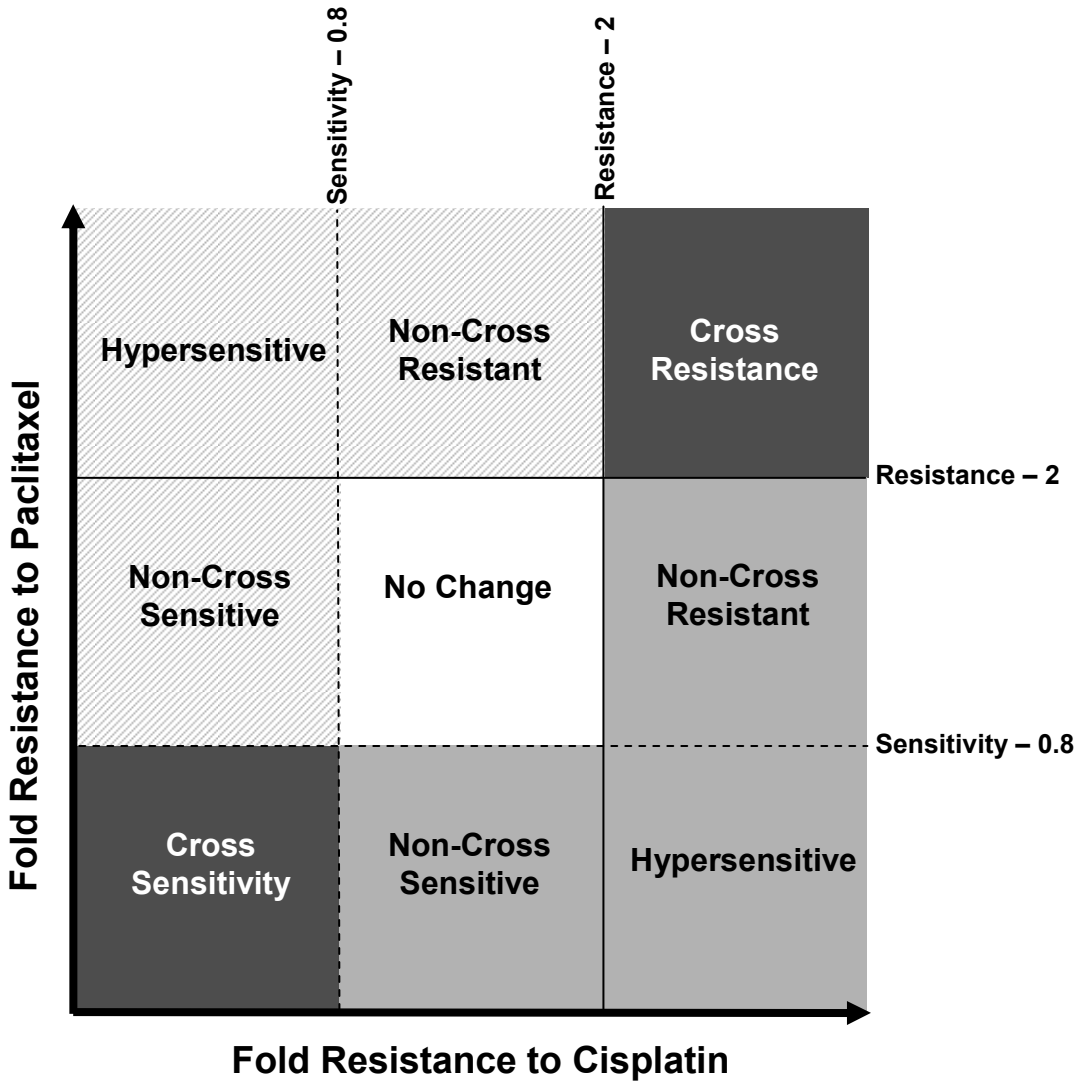

Figure 1 – Defining Resistance. Resistance is defined as a fold resistance of greater than or equal to 2 and sensitivity as a fold resistance of less than or equal to 0.8. Cross resistance is therefore greater than 2-fold to both drugs and cross sensitivity less than 0.8 to both drugs, and are indicated in black. Non-cross resistance is resistance to one drug with no change to the other compound. Hypersensitivity is resistance to one drug which has induced sensitivity to the other compound. Non-cross sensitivity is sensitivity to one drug and no change to the other. Grey areas indicates the categories that will aid our understanding of cisplatin resistance and paclitaxel sensitivity. Areas indicated with grey stripes are the opposite phenotype, paclitaxel resistance and cisplatin sensitivity.

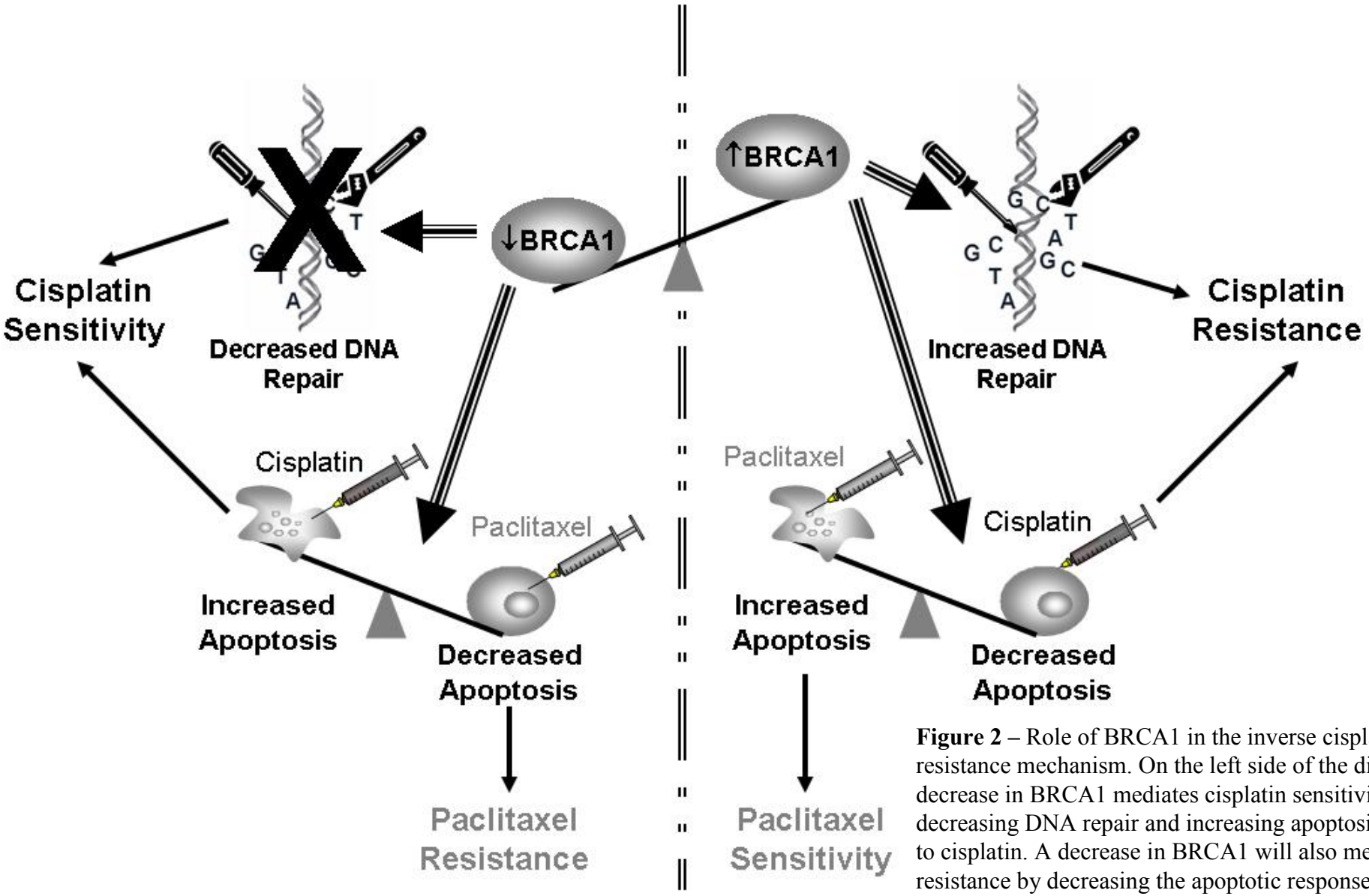

Figure 2 – Role of BRCA1 in the inverse cisplatin/paclitaxel resistance mechanism. On the left side of the diagram a decrease in BRCA1 mediates cisplatin sensitivity by decreasing DNA repair and increasing apoptosis in response to cisplatin. A decrease in BRCA1 will also mediate paclitaxel resistance by decreasing the apoptotic response to paclitaxel. On the right side of the diagram an increase in BRCA1 mediates cisplatin resistance by increasing DNA repair and decreasing apoptosis in response to cisplatin. An increase in BRCA1 will also mediate paclitaxel sensitivity by increasing the apoptotic response to paclitaxel.