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Genomic changes identified by comparative genomic hybridisation in docetaxel-resistant breast cancer cell lines

Sarah L. McDonald [a], David A.J. Stevenson [c], Susan E. Moir [a], Andrew W. Hutcheon [d], Neva E. Haites [c], Steven D. Heys [a], Andrew C. Schofield [a,b,*]

[a] *School of Medicine, College of Life Sciences and Medicine, University of Aberdeen, Medical School, Foresterhill, Aberdeen AB25 2ZD, UK*
[b] *School of Medical Sciences, College of Life Sciences and Medicine, University of Aberdeen, Medical School, Foresterhill, Aberdeen AB25 2ZD, UK*
[c] *Department of Medical Genetics, NHS Grampian, Aberdeen Royal Infirmary, Foresterhill, Aberdeen AB25 2ZD, UK*
[d] *Department of Medical Oncology, NHS Grampian, Aberdeen Royal Infirmary, Foresterhill, Aberdeen AB25 2ZD, UK*

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Abstract

Docetaxel is one of the most effective chemotherapeutic agents in the treatment of breast cancer. Breast cancers can have an inherent or acquired resistance to docetaxel but the causes of this resistance remain unclear. In this study high-level, docetaxel-resistant human breast cancer cell lines (MCF-7 and MDA-MB-231) were created, and comparative genomic hybridisation was used to identify genomic regions associated with resistance to docetaxel. MCF-7 resistant cells showed an amplification of chromosomes 7q21.11-q22.1, 17q23-q24.3, 18, and deletion of chromosomes 6p, 10q11.2-qter and 12p. MDA-MB-231 resistant cells showed a gain of chromosomes 5p, 7q11.1-q35, 9, and loss of chromosomes 4, 8q24.1-qter, 10, 11q23.1-qter, 12q15-q24.31, 14q and 18. Whole chromosome paints confirmed these findings. Amplification of 7q21 and loss of 10q may represent a common mechanism of acquired docetaxel resistance in breast cancer cells. This study is the first description of a genomic approach specifically to identify genomic regions involved in resistance to docetaxel.

Keywords: Breast neoplasms; Chromosome aberrations; *In situ* hybridisation fluorescence; Taxoids; Anti-neoplastic agents; Drug therapy; Drug resistance

1. Introduction

Docetaxel is one of the most effective chemotherapeutic agents used in the treatment of patients with breast cancer [1,2]. However, breast cancers may be either intrinsically resistant, or develop acquired resistance, to docetaxel as treatment occurs. Resistance to docetaxel is a major clinical problem, which limits its effectiveness in the treatment of patients with breast cancer. To date, the mechanisms of docetaxel resistance are poorly understood. Recent studies, however, have indicated that a limited number of genes may contribute towards resistance to docetaxel and another taxane, paclitaxel [3–9]. These genes can be categorised as either being those specifically involved in the anti-tumour mode of action of taxanes, or those involved commonly in resistance to many different chemotherapeutic agents.

Docetaxel exerts its effects by binding to β-tubulin subunits of microtubules. This results in preventing the depolymerisation of microtubules, blocking cell growth in the G2-M phase, and resulting in apoptosis [10]. Mutations in β-tubulin [3], the disruption of microtubule dynamics [4], and the differential expression of β-tubulin isoforms [5] in lung and breast tumours have been associated with resistance to docetaxel and paclitaxel. The second category of genes, involved in drug

* Corresponding author. Tel.: +44 1224 553006; fax: +44 1224 555199.
E-mail address: a.schofield@abdn.ac.uk (A.C. Schofield).

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resistance, encode proteins that are commonly involved in allowing resistance to occur to many other chemotherapeutic agents. For example, overexpression of drug transporters such as P-glycoprotein, which increases drug efflux from the cell [6] or drug metabolising enzymes such as CYP3A4 [7], oncogenes such as Src protein kinase [8] or tumour suppressor genes such as *BRCA2* [9] are all believed to be important in the resistance to several chemotherapeutic agents. However, such mechanisms do not account for resistance to chemotherapeutic agents in all tumours. It is important to identify the novel genes that may be involved in chemotherapy resistance to allow a more complete understanding of the mechanisms involved in docetaxel resistance.

The majority of previous studies investigating resistance to docetaxel and paclitaxel have adopted a candidate gene approach. Such an approach, which by its nature is focused on individual candidate genes, may fail to identify novel genes involved in mechanisms of drug resistance because it does not allow a global genomic assessment to be made. A novel genomic approach to understanding drug resistance is comparative genomic hybridisation (CGH), which has been used widely in cancer research to identify novel regions of genomic amplification and/or deletion [11,12]. CGH involves the hybridisation of different fluorescently labelled genomic DNA (from two different samples, e.g., tumour *versus* normal) to normal human metaphase chromosomes. Following hybridisation, the pattern of differential fluorescence along the length of each chromosome is visualised by fluorescent microscopy and analysed using computer software. Chromosomal regions, which are either gained or lost in genomic DNA of one sample compared with another, can be identified using this technique. The regions that are identified as being altered can then be searched to identify the genes, which may be involved in control of cellular growth. Some initial studies have applied a modified version of CGH to detect chromosomal imbalances specifically involved in the resistance to chemotherapeutic agents such as 5-fluorouracil, fluoropyrimidines and the platinums [13–16].

However, the genomic changes specifically involved in resistance to docetaxel have not previously been reported. This study aims to identify novel regions of genomic change involved in resistance to docetaxel by carrying out CGH in breast cancer cell lines made resistant to docetaxel.

2. Materials and methods

2.1. Cell culture

Human breast cancer cell lines, MCF-7 (oestrogen-receptor positive) and MDA-MB-231 (oestrogen-receptor negative), were obtained from the American Type Tissue Culture Collection (Manassas, VA, United States of America (USA)). The docetaxel-resistant sub-lines were generated by incubation in culture media supplemented with increasing concentrations of docetaxel (Aventis Pharma Ltd., West Malling, Kent, United Kingdom (UK)), as described previously [17].

2.2. Cytotoxic assay

Docetaxel resistance was demonstrated in the cell lines by means of the 3-(4-5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye reduction assay. The MTT assay was also used to determine the sensitivity of the cell lines to other chemotherapeutic agents: doxorubicin (Mayne Pharma Plc, Warwickshire, UK), 5-fluorouracil (Sigma, Gillingham, Dorset, UK), paclitaxel (Sigma) and vincristine (Sigma). Direct cell counts, using trypan blue solution, were also obtained to determine the concentration of docetaxel required to cause 50% inhibition of cell growth (known as the IC_{50} value), as described previously [17].

2.3. Comparative genomic hybridisation (CGH)

A modified CGH reaction was carried out as described previously [13], with the following amendments. CGH was performed using MCF-7 and the MDA-MB-231 cells and their docetaxel-resistant sub-lines. Genomic DNA was isolated from the cell lines using a QIAamp® DNA mini kit (Qiagen, Crawley, West Sussex, UK). DNA concentration was quantified by fluorometry (Sigma). Nick translation was performed to generate DNA fragments between 200 and 2000 bp long. The CGH reaction incorporated 800 ng Spectrum Red-labelled DNA (Abbott Laboratories, Maidenhead, UK) and 800 ng Spectrum Green-labelled DNA (Abbott Laboratories). To block repetitive DNA sequences, 100 times excess Cot-1 DNA (Abbott Laboratories) was used. The DNA was precipitated with 10% (w/v) 3 M sodium acetate (pH 7) and 2.5 times volume 100% ethanol. The DNA pellet was dried and re-suspended in hybridisation buffer (70% (v/v) formamide, 10% (v/v) dextran sulphate, 2 × SSC). Normal human metaphase chromosomes were obtained from lymphocyte cultures and the resulting slides were denatured at 75 °C for 5 min in denaturation solution (70% (v/v) formamide, 2 × SSC). The labelled DNA was denatured at 74 °C for 5 min before hybridisation with the metaphase spreads and incubated in a humidified incubator at 37 °C for 5 d. Post-hybridisation washes consisted of: 3 s in 0.4 × SSC, 0.3% (v/v) Igepal at 74 °C; and 3 s in 2 × SSC, 0.1% (v/v) Igepal at room temperature. The slides were briefly air dried and stained with 0.1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI). In order to confirm the genomic alterations observed, the experiments were repeated with reverse labelling. In

addition, the experiments were repeated in duplicate with DNA isolated from two independent extractions.

2.4. Digital image analysis

Metaphase chromosomes were captured with a Zeiss Axioplan II fluorescent microscope connected to a cooled charged coupled device camera (Photometrics, Waterloo, Canada). Three single colour images were taken for each metaphase using Spectrum Green, Spectrum Red and DAPI filters. The images were then processed using the Powergene Macintosh version 4.5 software (Applied Imaging, Newcastle, UK). At least 15–20 metaphases were captured per slide. Chromosomal regions were expressed as a gain if the green-to-red ratio was greater than 1.15 and losses were expressed as less than 0.85. A chromosomal region was identified as a genomic change if the same chromosomal region was altered in every replicate.

2.5. Fluorescent in situ hybridisation

Whole chromosome paints were obtained from Qbiogene (Livingston, UK) and denatured according to manufacturer's instructions. A Bcl-2 specific DNA probe (Qbiogene) was used according to manufacturer's instructions. Bacterial artificial chromosome (BAC) DNA probes, RP11-91M13, RP11-72J24 and RP11-89O20, mapping to chromosome 7q21.1, 7q22.2 and 7q31.1, respectively, were obtained from BAC PAC resources (California, USA). DNA was obtained and prepared as described previously [18]. BAC DNA probes were re-suspended in 10 μl CEP hybridisation buffer (Abbott Laboratories) and denatured at 96 °C for 5 min. The metaphase chromosome slides were prepared from the cell lines by incubating 70% confluent cells with 350 μl colcemid for 2 h 20 min, before trypsinising and fixing three times in 3:1 methanol:acetic acid. The resulting slides were denatured at 75 °C for 2 min in denaturation solution (70% (v/v) formamide, 2 × SSC), followed by the addition of the denatured BAC DNA probes to the denatured chromosomes, prior to incubating in a humidified incubator at 37 °C for 16 h. Post-hybridisation washes for the Bcl-2 probe consisted of: 2 min in 0.4 × SSC, 0.3% (v/v) Igepal at 74 °C; and 1 min in 2 × SSC, 0.1% (v/v) Igepal at room temperature. In contrast, whole chromosome paints and BAC DNA probes were washed for 15 s. The slides were briefly air dried and stained with 0.1 μg/ml DAPI. The specificity of all DNA probes was checked by hybridisation to normal human metaphase chromosomes.

2.6. Western analysis for P-glycoprotein expression

Western analysis was carried out as described previously [17], with the following amendments. Ten micrograms of protein was electrophoresed through a precast 7.5% tris–glycine polyacrylamide gel (Cambrex Bioscience, Nottingham, UK). Each membrane was incubated with 1:200 dilution (in 5% (w/v) milk/Tris-buffered saline with 0.1% (v/v) Tween 20) of mouse monoclonal anti-human MDR-1 antibody (Autogen Bioclear, Calne, Wiltshire, UK) or 1:5000 β-actin (Abchem, Cambridge, UK) for 1 h at room temperature. The experiments were repeated in triplicate with protein isolated from two independent extractions.

3. Results

3.1. Confirmation of resistance to docetaxel in the developed docetaxel-resistant sub-lines

Firstly, the *in vitro* effect of docetaxel on cell growth was determined using a standard cell viability assay. This demonstrated that docetaxel was more cytotoxic in MCF-7 and MDA-MB-231 cells than in their resistant sub-lines (MCF-7 TAX30 and MDA-MB-231 TAX30, respectively), which had acquired resistance following sequential exposure to a maximum 30 μmol/l docetaxel over a period of 50 weeks (Fig. 1). The MTT assay demonstrated that even at 30 μmol/l docetaxel, there was still an MTT activity of 50% in the parental MCF-7 cells. However, this may be related to a possible biphasic response to docetaxel, but it is also well recognised that docetaxel can cause an increase in absorbance thus giving falsely elevated absorbance values [19]. Therefore, direct cell counts were carried out in order to determine the 50% inhibitory concentrations (IC_{50}) for docetaxel in the breast cancer cell lines. This method allows a more accurate determination of the number of viable cells compared with the MTT assay. The IC_{50} of docetaxel in MCF-7 cells was 0.015 μmol/l, whereas the MCF-7 TAX30 sub-lines had an IC_{50} value of 10 μmol/l. The IC_{50} of docetaxel in MDA-MB-231 cells was 0.04 μmol/l, whereas the MDA-MB-231 TAX30 sub-lines had an IC_{50} value of 55 μmol/l. Furthermore, MCF-7 TAX30 and MDA-MB-231 TAX30 resistant sub-lines exhibited cross-resistance to paclitaxel, vincristine and doxorubicin, but did not demonstrate cross-resistance to 5-fluorouracil (data not shown).

3.2. Genomic regions associated with docetaxel resistance

CGH was used to identify genomic regions modified as a result of acquired drug resistance to docetaxel (Fig. 2). A common region of amplification of chromosome 7q and loss of chromosome 10q were identified in both MCF-7 and MDA-MB-231 docetaxel-resistant sub-lines (Fig. 3). MCF-7 TAX30 sub-lines exhibited a gain of chromosome 18, however, chromosome 18 was

lost in MDA-MB-231 TAX30 sub-lines (Fig. 3). Additional genetic alterations, distinct to each docetaxel-resistant cell line, were also identified (Table 1). Whole chromosome paints confirmed the presence of genetic aberrations that had initially been detected by CGH (Fig. 4). A specific DNA probe to Bcl-2 (which is a gene encoded on chromosome 18q21.33) was used to investigate the genetic alteration of chromosome 18. The DNA probe for Bcl-2 was amplified in MCF-7 TAX30 sub-lines (three hybridisation signals compared with two signals in MCF-7 cells), whereas the Bcl-2 probe signal was lost in MDA-MB-231 TAX30 sub-lines (one hybridisation signal compared with two signals in MDA-MB-231 cells). The Bcl-2 probe thus confirmed the CGH results for the genetic alterations on chromosome 18.

3.3. Confirmation of chromosome 7q genomic amplification in docetaxel-resistant breast cancer cells

CGH identified numerous genetic alterations in docetaxel-resistant sub-lines. The next stage, therefore, was to localise the genomic regions further to identify candidate genes involved in resistance. Since chromosome 7q demonstrated the greatest amplification peak,

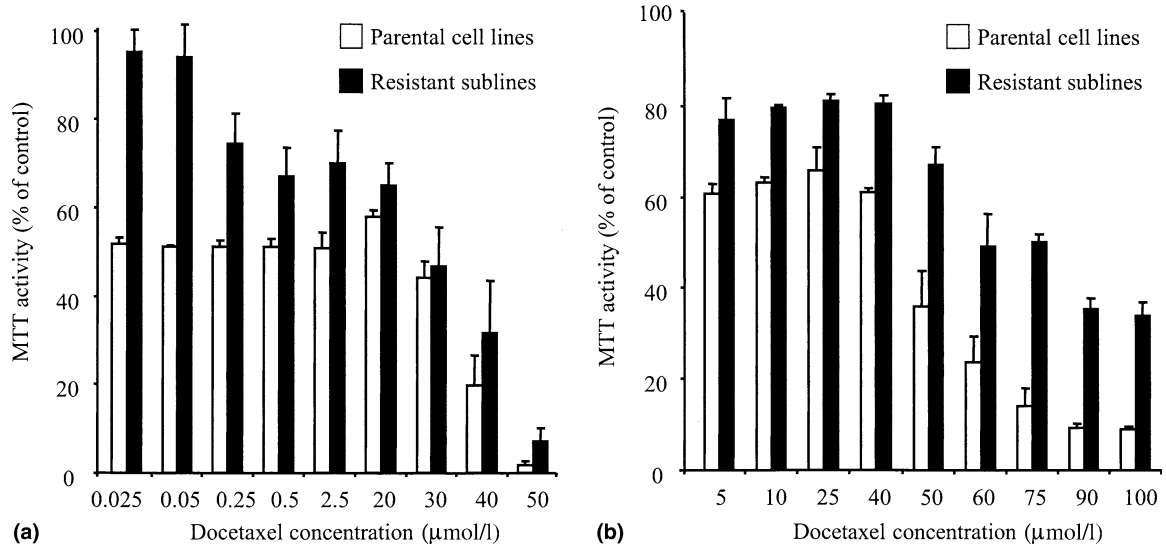

Fig. 1. Demonstration of sensitivity to docetaxel between parental breast cancer cells and resistant sub-lines, which had acquired resistance during sequential exposure to a maximum 30 μmol/l docetaxel. (a) MCF-7 breast cancer cells; (b) MDA-MB-231 breast cancer cells. MTT, 3-(4-5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

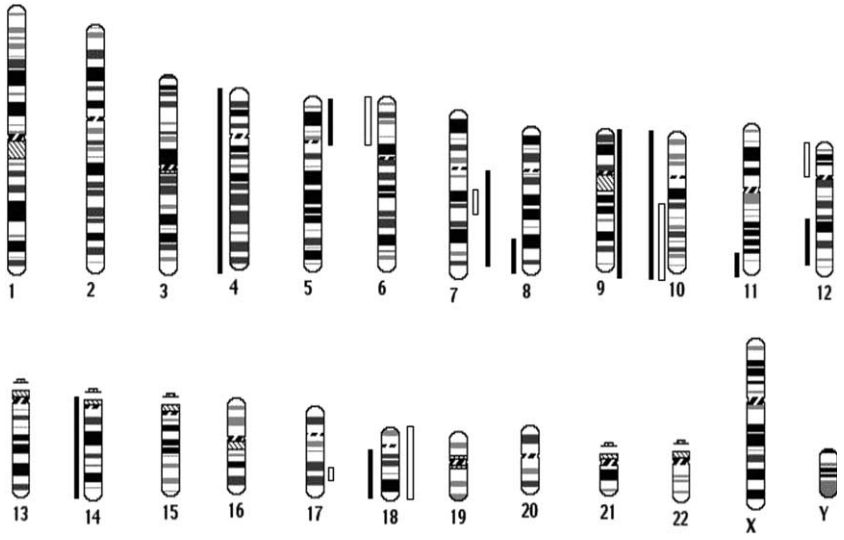

Fig. 2. Cytogenetic location of genetic alterations associated with docetaxel resistance. Chromosomal alterations identified in MCF-7 TAX30 cells are indicated by open bars and alterations identified in MDA-MB-231 TAX30 cells are indicated by filled bars. A bar towards the right of the chromosome indicates regions of gain, a bar towards the left indicates genomic losses.

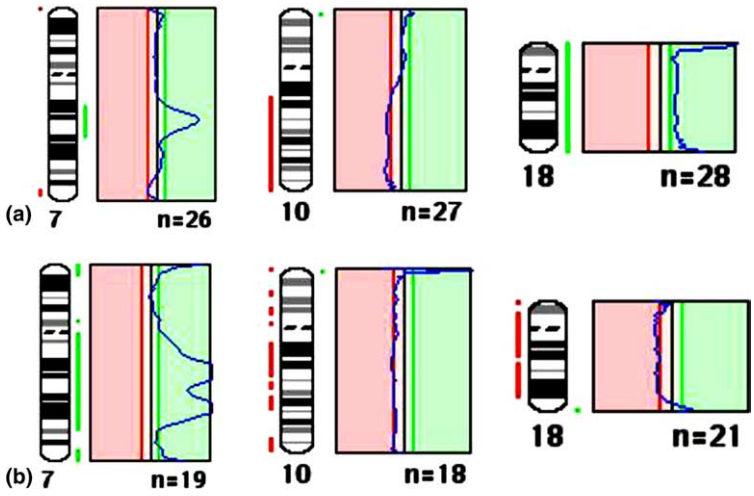

Fig. 3. Genomic imbalances detected by comparative genomic hybridisation (CGH) in docetaxel-resistant sub-lines. (a) MCF-7 TAX30 resistant cells; (b) MDA-MB-231 TAX30 resistant cells. A CGH profile towards the right indicates regions of gain, a profile towards the left indicates genomic losses.

Table 1
Genetic alterations associated with docetaxel resistance in breast cancer cells

Cell line	Gain	Loss
MCF-7 TAX30	7q21.11-q22.1 17q23.3-q24.3 18p 18q	6p 10q11.2-qter 12p
MDA-MB-231 TAX30	5p 7q11.1-q35 9p 9q	4p 4q 8q24.1-qter 10p 10q 11q23.1-qter 12q15-q24.31 14q 18p 18q

as detected by CGH, it was decided to focus on this chromosomal region. Fluorescent *in situ* hybridisation was carried out with BAC DNA probes to confirm the CGH-detected amplification of chromosome 7q in docetaxel-resistant sub-lines.

MCF-7 TAX30 resistant cells displayed an amplification of chromosome 7q21.11-q22.1. MCF-7 cells showed no amplification, using the BAC DNA probe, RP11-91M13 (mapping to the cytogenetic band 7q21.1), however, there was a high level of amplification seen in the MCF-7 TAX30 resistant sub-lines, thus supporting the CGH results. When the analysis was carried out on metaphase chromosomes, it was apparent that the signals were condensed to one chromosome region. In addition, BAC DNA probes, RP11-72J24 and RP11-89O20 (mapping to the cytogenetic band 7q22.2 and 7q31.1, respectively), which both lie outside the CGH amplified region in MCF-7 TAX30 resistant cells, were not amplified.

MDA-MB-231 TAX30 resistant cells exhibited two distinct bands of amplification at chromosome 7q21 and 7q31. Using BAC DNA probes mapping specifically to these two chromosomal regions, 7q21.1 (RP11-91M13) and 7q31.1 (RP11-89O20), confirmed the CGH amplified regions in MDA-MB-231 TAX30 resistant cells. Furthermore, a BAC DNA probe, RP11-72J24 (7q22.2), mapping between the two CGH amplified regions, was not amplified in MDA-MB-231 TAX30 resistant cells. BAC DNA probes, therefore, confirmed the amplification of chromosome 7q, detected by CGH, and support the use of CGH as an application to identify genomic aberrations.

3.4. P-glycoprotein overexpression is associated with docetaxel resistance

CGH identified a common region of chromosome 7q to be amplified in both docetaxel-resistant cell lines. Furthermore, this amplification was confirmed using BAC DNA probes mapping specifically to the region. The next step was to determine whether the amplified genomic region was associated with protein overexpression. A candidate gene encoded within this amplified region was MDR-1, as increased expression of the protein product of this gene, P-glycoprotein, is a well-recognised multi-drug resistance mechanism. Western analysis was carried out and this revealed that P-glycoprotein was overexpressed in both docetaxel-resistant sub-lines (Fig. 5).

3.5. Effects of inhibition of P-glycoprotein (by verapamil) on resistance to docetaxel in docetaxel-resistant breast cancer cell lines

In order to determine whether P-glycoprotein was involved in resistance, the level of resistance to docetaxel

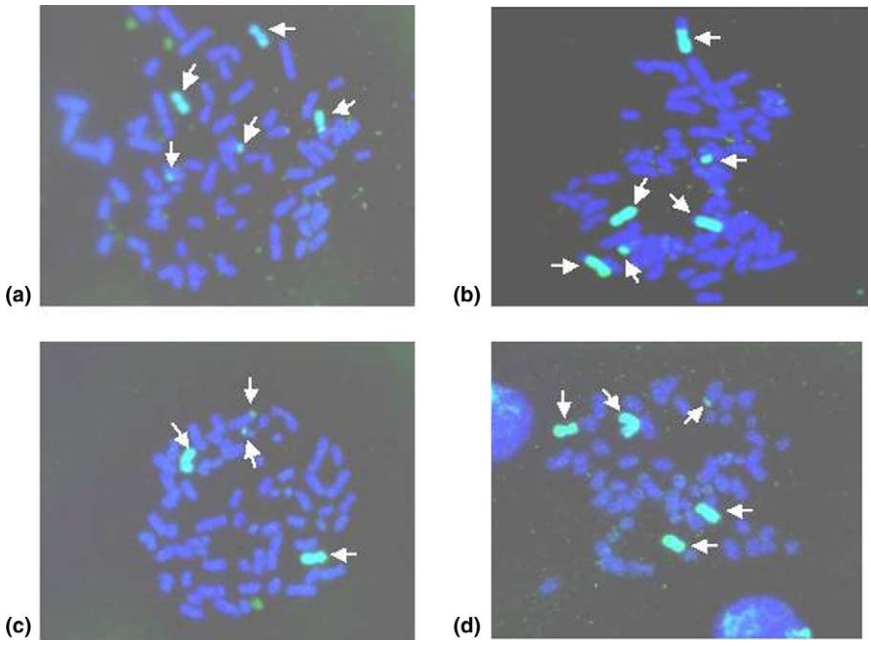

Fig. 4. Chromosome 7 paint confirms amplification of chromosome 7 material in docetaxel-resistant sub-lines. (a) MCF-7; (b) MCF-7 TAX30; (c) MDA-MB-231; (d) MDA-MB-231 TAX30.

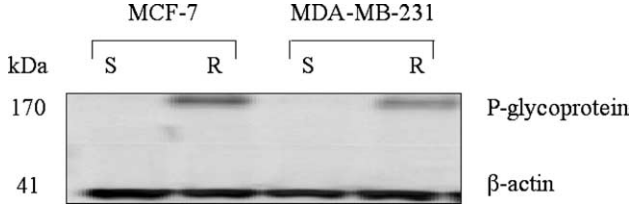

Fig. 5. P-glycoprotein is overexpressed in docetaxel-resistant cells. Total protein was separated by polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes and probed with mouse monoclonal anti-human MDR-1 antibody. S, parental breast cancer cells; R, docetaxel-resistant sub-lines; kDa, kiloDaltons.

in the resistant breast cancer cell lines was measured in the presence of the calcium-channel blocker, verapamil, which is known to inhibit the function of P-glycoprotein [20]. The *in vitro* effect of docetaxel and verapamil on cell growth of the docetaxel-resistant breast cancer cell lines was determined using a standard cell viability assay. Verapamil (10 μmol/l) demonstrated greater docetaxel-induced cytotoxicity at lower concentrations of docetaxel (less than 0.05 μmol/l) in both resistant cell lines (Fig. 6). When 100 μmol/l verapamil was used, however, resistance was only notably reversed at higher

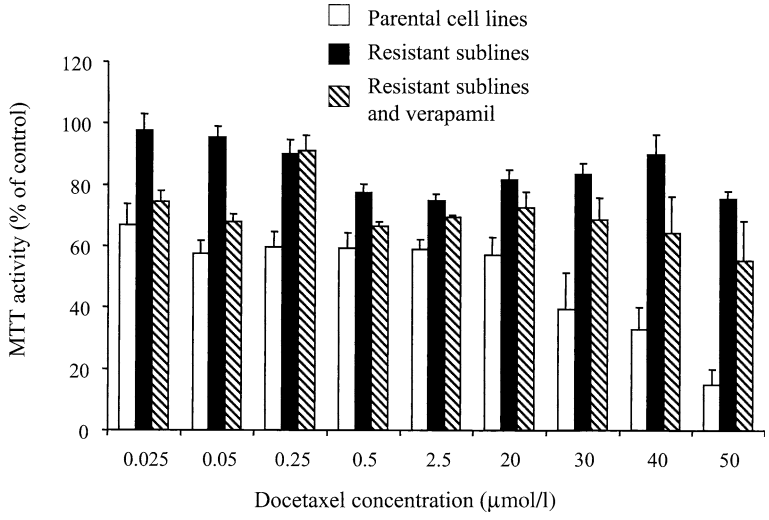

Fig. 6. Effect of docetaxel and verapamil on cellular viability. Demonstration of increased sensitivity to docetaxel in MDA-MB-231 TAX30 sub-lines cultured with 10 μmol/l verapamil. Verapamil had the greatest effect on docetaxel-induced cytotoxicity at lower concentrations of docetaxel. MTT, 3-(4-5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

concentrations of docetaxel (75–100 μmol/l). These results demonstrate that a P-glycoprotein inhibitor, verapamil, enhances docetaxel-induced cytotoxicity in docetaxel-resistant cells at specific concentrations.

4. Discussion

This study is the first global analysis of the genetic events involved in resistance to docetaxel, where a high-level of resistance to docetaxel has been created. We identified a common amplified region of chromosome 7q21 and loss of chromosome 10q in two breast cancer cell lines, during the development of acquired resistance to docetaxel. In addition, both docetaxel-resistant sub-lines also exhibit different genetic modifications. This demonstrates that resistance to docetaxel is a multi-factorial phenomenon with many different genes contributing to the resistant phenotype.

A similar study using MCF-7 breast cancer cell lines, resistant to paclitaxel, demonstrated amplification of chromosome 7q [21]. It seems plausible, therefore, that chromosome 7q amplification may be a common event in the resistance to all taxanes. A candidate gene encoded within this amplified region was MDR-1, as increased expression of the protein product of this gene, P-glycoprotein, is a well-recognised multi-drug resistance mechanism. We demonstrated that the expression of P-glycoprotein is increased in both docetaxel-resistant sub-lines when compared with the docetaxel-sensitive parental cell lines. The identification of P-glycoprotein overexpression is important not just because it highlights a mechanism of resistance, but because it demonstrates that a gene encoded within an amplified chromosome region, detected by CGH, is involved in the generation of the resistant phenotype in these breast cancer cell lines.

In terms of chemotherapy resistance, however, this was only partially reversed by the addition of verapamil (an inhibitor of P-glycoprotein). Complete reversal of resistance still could not be achieved by increasing the concentration of verapamil even though a previous study has shown verapamil, at a concentration of 1 μmol/l, to almost completely reverse resistance in human pancreatic cancer cells, which had been made resistant to docetaxel [22]. The extent of the contribution of P-glycoprotein towards chemotherapy efflux is influenced by the extracellular concentration of the chemotherapeutic agent. P-glycoprotein-mediated efflux of paclitaxel in BC19 human breast cancer cells is maximal at concentrations less than 200 nmol/l [23]. The authors noted that higher concentrations of paclitaxel decreased the rate of P-glycoprotein-mediated efflux.

If P-glycoprotein-mediated efflux of docetaxel is similarly influenced at different drug concentrations, this may explain why reversal of resistance in our docetaxel-resistant breast cancer cell lines was greater at certain concentrations of docetaxel. Verapamil may only reverse resistance in breast cancer cells, which are overexpressing P-glycoprotein, at concentrations of docetaxel that are compatible with a P-glycoprotein-mediated efflux mechanism. A different drug resistance mechanism may be involved in breast cancer cells that have a different extracellular concentration of docetaxel. Our results, therefore, suggest that while increased expression of P-glycoprotein may be involved in resistance to docetaxel, it may not be the only mechanism involved.

It is possible that other genes contained within the amplification of chromosome 7 may be involved in docetaxel resistance. For example, the cytochrome P450 enzymes CYP3A4, CYP3A43, CYP3A7 and CYP3A5 are located on chromosome 7q22.1. CYP3A4 is responsible for metabolising docetaxel into an inactive precursor, and is amplified in breast tumours that demonstrate a poor clinical response to docetaxel [7]. Other genes of interest in the region are the heat-shock proteins, *HSPB1*, *HSPC047* and *DNAJB9*, since overexpression of some of these protein family members has been shown to be involved in resistance to other chemotherapeutic agents, including paclitaxel and cisplatin [24,25]. Altered expression of genes involved in cell cycle regulation and intracellular signalling pathways have been identified in breast tumours of patients who demonstrated a poor response to docetaxel therapy [26]. There are several genes located on chromosome 7q, such as *DMTF1*, *CDK6*, *YWHAG* and *PTPN12*, which are involved in cell cycle regulation and signalling processes. Although there are many genes located in this region, at the present time these have not been linked to chemotherapy resistance. Indeed, this emphasises the usefulness of a global approach, such as CGH, as this may indicate the possible involvement of other genes, which hitherto have not been linked to the development of resistance to chemotherapeutic agents and can then be explored in this respect.

Both docetaxel-resistant cell lines exhibited a loss of chromosome 10q. This chromosomal region has not previously been reported to be involved in resistance to either docetaxel or paclitaxel. However, loss of the *PTEN* tumour suppressor gene, encoded on chromosome 10q23.31, has been implicated in resistance to other chemotherapeutic agents, such as doxorubicin, in bladder and prostate cancer [27,28]. Furthermore, if the expression of PTEN protein is increased, by transfection of *PTEN*, this enhances doxorubicin-induced cytotoxicity in PTEN-negative PC-3 prostate cancer cells [28]. There are other genes of interest on chromosome 10q including other tumour suppressor genes (e.g., *TRIM8*, *TACC2*) and genes involved in regulating the apoptotic pathway (e.g., *BINP3*, *BAG3*). Docetaxel resistance may be acquired by loss of these genes, which may lead to a more aggressive cell type, or may lead to the cells escaping drug-induced apoptosis. These genomic changes,

however, remain to be validated in order to identify specific genes associated with docetaxel resistance.

A gain of chromosome 18 was observed in the MCF-7 resistant sub-lines whilst a region of it was lost in the MDA-MB-231 resistant sub-lines. The gene for the anti-apoptosis protein Bcl-2 maps to this region. We confirmed that Bcl-2 was amplified in MCF-7 docetaxel-resistant breast cancer cells but, in contrast, is deleted in MDA-MB-231 resistant cell lines. This may be important because regulation of Bcl-2 has been shown to be an important process involved in the cellular response to taxanes [29]. Modulation of Bcl-2 expression may present a mechanism by which cells become resistant to docetaxel. This may initially seem surprising since the chromosomal region containing Bcl-2 is both gained and lost, depending upon the type of docetaxel-resistant cell line. However, the paradoxical role of Bcl-2 has previously been shown with response to paclitaxel but not docetaxel. Overexpression of Bcl-2 is associated with resistance to paclitaxel in myeloma cells [30]. In contrast, reduced expression of Bcl-2 has been associated with resistance to paclitaxel in A2780 human ovarian cancer cell lines [31]. It is possible that the docetaxel-resistant sub-lines have acquired different mechanisms to control the apoptotic pathway and prevent chemotherapy-induced cytotoxicity.

The genes contained within the other genomic regions could exert their effects by a number of mechanisms. These include increased expression of drug transporters (ABCA1, CFTR), increased expression of enzymes involved in metabolising docetaxel (CYP3A4), and aberrant expression of proteins involved with microtubule dynamics (TUBB4Q, EMAP), regulators of the cell cycle (CDKN1A, NEK6, CHEK1) and apoptotic pathways (CASP3, BAK, BCL-2).

During this study, we used CGH to detect regions of genomic aberrations associated with docetaxel resistance. However, CGH has limitations, and has only been shown to detect regions of amplification larger than 5 Mb [32] and regions of deletion greater than 10 Mb [33]. Small genomic aberrations, therefore, will remain undetected and the genomic regions identified by CGH may contain many genes. Furthermore, the results obtained from our study depend on the use of well-established human cancer cell lines as a suitable *in vitro* model of drug resistance. A consideration when using cell lines to study the biology and behaviour of cancer cells *in vitro* is that prolonged culture may result in modifications to genetic and phenotypic stability [34,35]. Therefore, we have cultured resistant and sensitive cells in parallel, and limited the number of passages to try to minimise these effects and we avoid prolonged culture.

The molecular mechanisms involved in drug resistance *in vitro* may differ from the mechanisms involved with *in vivo* drug resistance, which may be due to differences in drug administration [36]. Furthermore, our model utilises cells that have a high-level of acquired resistance to docetaxel, which represents a specific situation. A previous study, however, demonstrated a good correlation between genomic aberrations identified in cancer cell lines with those found in tumours [37]. Another important study has shown that the patterns of gene expression in breast cancer cells *in vitro*, exposed to chemotherapeutic agents, was similar to those observed in patients with breast tumours treated with these drugs [38]. A more detailed and accurate assessment of the genes involved in drug resistance could be obtained using microarray expression analyses or proteomics. A recent study, however, combining CGH and RNA microarrays, demonstrated that 60% of the genomic changes identified by CGH correlated directly with changes in mRNA expression [39].

This is the first description of a genomic approach to identify changes specifically involved in acquired resistance to docetaxel in breast cancer cells. We have demonstrated that one of the genes contained within the amplified region may contribute to the resistant phenotype. It is therefore feasible that genes contained within other genomic regions may also be involved. The genetic regions identified will be investigated further to identify which genes play a key role in docetaxel resistance. Understanding the genetic events involved in resistance to chemotherapy may allow further development in modulating drug resistance, and may be important in allowing selection of appropriate patients who are most likely to benefit from such therapies.

Conflict of interest statement

None declared.

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