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Biophysical characterization of MDR breast cancer cell lines reveals the cytoplasm is critical in determining drug sensitivity

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Abstract

Dielectrophoresis (DEP) was used to examine a panel of MCF-7 cell lines comprising parental MCF-7 cells and MDR derivatives: MCF-7TaxR (paclitaxel-resistant, P-glycoprotein (P-gp) positive), MCF-7DoxR (doxorubicin-resistant MRP2 positive) plus MCF-7*MDR1* (*MDR1* transfected, P-gp positive). MCF-7DoxR and MCF-7*MDR1* were broadly cross-resistant to natural product anticancer agents, whereas MCF-7TaxR cells were not, contrary to P-gp expression. Whilst DEP revealed modest membrane changes in MDR sub-lines, we saw significant changes in their cytoplasmic conductivity: MCF-7TaxR < MCF-7 < MCF-7MDR1 < MCF-7DoxR (range 0.14–0.40 S/m). Cytoplasmic conductivity is affected by the movement of molecules e.g. as in intracellular trafficking MCF-7TaxR showed a reduced membrane potential, whereas MCF-7DoxR and MCF-7*MDR1* showed an increase. Thus, altered membrane potential is associated with an MDR phenotype, but in a complex manner. DEP data suggest a model whereby relative increases in cytoplasmic conductivity are correlated with MDR, whilst relative decreases equate with a sensitised phenotype e.g. MCF-7TaxR. Moreover, extent of anthracycline accumulation was inversely related to cytoplasmic conductivity. These data are representative of a model where drug sensitivity is associated with low ionic conductance (reduced cellular trafficking and ion transport) and substantial anthracycline accumulation. For classical MDR i.e. MCF-7*MDR1*, we saw the reverse picture. Thus, the drug resistance phenotypes of this panel of MCF-7 lines can be delineated by assessment of cytoplasmic biophysical properties using DEP.
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1. Introduction

It is a number of years since the first description of anticancer drug resistance [1], and since then many mechanisms have been described that attempt to explain the basis underlying this phenomenon. By far the most studied form of anticancer drug resistance is that of multidrug resistance (MDR), and findings from that research influenced to some extent research into MDR in microorganisms e.g. malaria and bacteria. Indeed, the cloning of *MDR* genes overexpressed in MDR tumour cells resulted in the discovery of a large family of membrane located glycoproteins—the *A*TP-*b*inding *c*assette (ABC) transporter proteins. The large majority of studies have focused on MDR1 protein, also referred to as P-glycoprotein (P-gp; recently renamed ABCB1).

Previous work by Wadkins and Roepe and others [2] went to some lengths to try to understand mechanisms that give rise to the MDR phenotype. For example, alterations in the membrane potential and also alkalinisation of the cytoplasm were shown to be features of P-gp expressing cells that could go some way towards explaining their drug resistant phenotype [3,4]. In this paper we set out to explain on the basis of biophysical parameters, the drug sensitivities of a panel of novel MCF-7 drug resistant cell lines: MCF-7TaxR, MCF-7DoxR and MCF-7*MDR1* compared with parental MCF-7 cells. In terms of their molecular characterisation, we show that MCF-7TaxR expresses P-gp *but* unusually it fails to demonstrate a broad cross-resistance (or multiple resistance) to natural product anticancer agents. The paclitaxel resistance in that cell line is due to P-gp overexpression as the modulating agent XR9576

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(P-gp specific) completely reverses the resistance. On the other hand, MCF-7DoxR being P-gp negative but broadly cross-resistance to many natural product anticancer agents in line with an MDR phenotype was shown to be weakly MRP2 (ABCC2) positive. MCF-7*MDR1* is a classical MDR cell line showing both membrane P-gp overexpression and cross-resistance to natural product anticancer agents [5,6].

It has been shown that cells transfected with MDR cDNA without subsequent maintenance in the presence of chemotherapeutic agents can certainly mimic some of the features of the MDR phenotype [7]. However, the level of drug resistance seen in those cells is somewhat less than that seen in drug-selected lines with comparable levels of P-gp expression. Moreover, in terms of understanding the biophysical events associated with MDR it is clear that data obtained using 'pure' transfected models are somewhat at variance with those obtained using cells cultured in a drug containing environment. We have focused on the latter as our model system — which used a panel of cell lines including an MCF-7*MDR1* transfected line but also maintained in a drug conditioned environment and, hence, not representative of a 'pure' transfection model. In terms of what is entirely relevant to the problem of clinical drug resistance and, hence, the ideal model has yet to be agreed upon by the cancer research community. Whilst it is imperative to define the effect of the *MDR1* gene expression itself on anticancer drug resistance, it is also important to understand those processes that bring about a drug-induced MDR phenotype. Acquired MDR phenotypes are well-documented phenomena and are highly relevant to the development of clinical drug resistance. Elegant studies such as those by Hoffman et al. [7] have been very important in helping us to understand a more precise role for *MDR1* in anticancer drug resistance. Notwithstanding, we propose that using cell line models with acquired drug resistant phenotypes obtained by growing up in a drug containing environment must bear some relation to the heavily pre-treated tumours that present with MDR in the clinic. Criticism of use of these types of cell line models has focused on the very high levels of drug resistance seen (sometimes in excess of 1000-fold) which have been deemed by many to be totally irrelevant to the problem of clinical drug resistance where 2-fold resistance to an anticancer agent will result in disease progression and kill the patient. We have used cell lines with relatively modest levels of drug resistance, maintained using drug doses relevant to clinically achievable blood levels.

Primarily, we have used the technique of dielectrophoresis (DEP) to understand the biophysical nature of the MCF-7 cell line panel, including the parental cells. DEP is a technique whereby the frequency-dependent behaviour of cells is determined in non-uniform electric fields, and from this information regarding the electrical properties of the cell can be inferred [8–10]. DEP and related techniques have been used to characterise a wide range of cellular processes in terms of these biophysical properties in cancer research [11–18]. Our studies have also incorporated a flow cytometric technique to measure membrane potential as an additional biophysical parameter to be considered in our assessment of the cell line panel. Previously we have shown that due to the nature of the putative drug efflux mechanism employed by MDR cells, dye-based methods of analysis (e.g. using DIOC5) to assess membrane potential can yield misleading results [19]. However, by combined use of an MDR modulating agent that corrects for defective DIOC5 accumulation due to ABC transporter efflux activity, we can achieve a more realistic interpretation of membrane potential in an MDR cell line. We have also looked at the cellular accumulation of anthracyclines in our MCF-7 panel of lines, using fluorescence microscopy. These data have highlighted a close association between cytoplasmic conductivity and extent of drug accumulation in the present study.

We demonstrate that there are significant changes in the cytoplasmic conductivity of all MDR cell lines relative to the parent line and also to each other. Interestingly, there are membrane changes measured using DEP seen for the paclitaxel-resistant line in terms of the membrane capacitance (C_{spec}) and not observed for either the doxorubicin-resistant or *MDR1* transfected MCF-7 lines. In addition, in line with its overall drug sensitivity profile, the MCF-7TaxR cell line is proficient in anthracycline accumulation, which is counterintuitive to the presence of membrane P-gp. Based on the present data, we can propose a model based on biophysical factors that can explain MDR whether in the absence or presence of Pgp or other ABC transporters.

2. Materials and methods

2.1. Chemicals and reagents

Paclitaxel (Taxol; obtained from Bristol Myers Squibb UK as a pharmacy preparation) and doxorubicin (Sigma Aldrich, Poole, UK) were dissolved in either sterile 0.9% NaCl solution (paclitaxel) or in sterile distilled water (doxorubicin). They were stored frozen as stock solutions and were thawed prior to use. DIOC5(3) (3,3′-dipentyloxacarbocyanine iodide; obtained from Molecular Probes, Invitrogen, Paisley, UK) was made up as an aqueous stock solution and stored at −20 °C until use. XR9576 (kindly provided by Xenova PLC, Slough, UK) was made up as a stock solution in DMSO and stored frozen until use. PSC-833 (Valspodar; obtained from Novartis, Basel, Switzerland) was made up as a 5 mM stock solution in ethanol, stored at −20 °C and then diluted accordingly for use in experiments.

2.2. Cell culture

All cell culture reagents were obtained from Sigma Aldrich (Poole, UK), unless stated otherwise. The MCF-7 human breast cancer cell line and its paclitaxel and doxorubicin resistant counterparts MCF-7TaxR and MCF-7DoxR, respectively were grown in 20 mM HEPES modified Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated foetal calf serum (FCS), (Invitrogen, Paisley, UK) and 2 mM L-glutamine. The cells were grown under standard incubation conditions at 37 °C. Both the drug resistant cell lines, MCF-7TaxR and MCF-7DoxR, were derived in our laboratories by growing up cells in standard culture conditions in increasing step-wise concentrations of the inducing agent over a period of approximately 3 months until a stable resistance phenotype was achieved. MCF-7TaxR was maintained in the presence of 6 nM paclitaxel, while MCF-7DoxR was maintained in the presence of 100 nM doxorubicin. The MCF-7*MDR1* cell line, kindly provided by Professor Robert Clarke, Georgetown University, Washington DC, US, was stably transfected with the *MDR1* gene and maintained with selection pressure using 200 ng/ml colchicine [20] The parental MCF-7 breast cancer cells were obtained from The European Collection of Cell Culture, Porton Down, Salisbury, UK (ECACC). All cultures were regularly checked for mycoplasma infection.

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