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Multidrug Resistance in Breast Cancer: Mechanisms, Strategies

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INTRODUCTION

BREAST CANCER is the leading cause of death among women in the Western world, where, despite radical surgery, approximately 35% of affected women die [1]. Although chemotherapy improves survival in the adjuvant setting, in metastatic disease, despite a response rate of over 50% in treated patients, development of drug resistance invariably occurs leading to untreatable disease. Moreover, despite the use of adjuvant treatment, around 50% of all patients will eventually relapse [2]. In the clinical setting it is unknown whether drug resistant clones develop under the pressure of chemotherapy, or if they were already present at the start of the treatment which they survive.

Among the most extensively studied mechanisms of drug resistance is multidrug resistance (MDR) which is due to active extrusion of drugs from the tumour cells (Table 1). Classical MDR includes crossresistance to natural product anticancer drugs (anthracyclines, epipodophyllotoxins, vinca alkaloids, actinomycin D and the taxoids) *in vitro* and is associated with overexpression of Pgp, a plasma membrane protein of 170 kDa molecular weight, encoded by the *MDR1* gene [3]. Pgp acts as an energy-dependent drug efflux pump, thereby decreasing the intracellular drug accumulation [3]. An interesting feature of Pgp-mediated MDR is that it can be reversed *in vitro* and also *in vivo* by several agents [3]. Several of these agents have been tested in haematological malignancies and solid tumours, and sufficient plasma concentrations, able to revert resistance *in vitro*, appear to be achievable with the most recently introduced drugs (e.g. cyclosporin and PSC 833), with relatively tolerable side-effects [4].

From a limited number of studies, there is evidence that Pgp might play a role in resistance to cytotoxic drugs used in breast cancer [5–8]. A correlation has been observed between Pgp expression in breast cancer cells obtained from patients and *in vitro* resistance to doxorubicin [5–7]. Furthermore, a high Pgp expression in 17 locally advanced breast cancer patients was associated with the lack of response to neoadjuvant chemotherapy and a shorter disease-free survival [8]. Although these results are of interest, the evaluation of Pgp expression as a prognostic factor still requires much larger data sets.

Table 1. Types of multidrug resistance

Type	Accumulation defect	Reversal
Pgp-mediated	Yes	Possible
MRP-mediated	Sometimes	Unknown
LRP-mediated	Sometimes	Unknown
topo-II-mediated	No	Unknown

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Recently, other mechanisms have been identified that can induce MDR in tumour cells. These have been identified in cell lines which display MDR, and which often do not have reduced drug uptake. These have been called atypical MDR and can be due to several factors other than Pgp overexpression. One factor is associated with alterations in the topoisomerase II enzyme activity. The products of two other very recently discovered genes, *MRP* (multidrug resistance associated protein) and *LRP* (lung resistance associated protein), are possibly also implicated in transport mechanisms.

Topoisomerases are ubiquitous enzymes, which are present in the nuclei of mammalian cells, and regulate the topological state of DNA. Essentially two major forms of topoisomerases have been described, topoisomerase I (topoI) and topoisomerase II (topoII) [9, 10]. There are several differences between the two enzymes, one being that topoI cuts one strand of DNA allowing the other strand to pass through the transient break, and, therefore, changes in the conformation of the DNA helix occur.

Alternatively, topoII cuts both strands of DNA simultaneously, allowing double-stranded DNA to go through the break. Both enzymes are able to reseal the DNA break after the passage has occurred. Changes in DNA topology are essential for several physiological cellular functions such as RNA transcription, DNA synthesis, replication, chromosomal segregation, recombination and others, among which possibly is DNA repair. While topoI expression and activity appear to be relatively uninfluenced by the proliferative status of the cell, topoII is highly sensitive to changes in the growth of cells and appears to be most active in phases preceding the S-phase of the cell cycle [11]. Recently an isoform of topoII, topoII-β, has been discovered. Its activity is relatively insensitive to proliferation changes compared to the first enzyme described, the topoII-α form [12–17].

Topoisomerases are important enzymes for antineoplastic therapy as they are targets of several antineoplastic agents long utilised in solid tumour and haematological malignancy regimens [11, 18, 19]. Anthracyclines, epipodophyllotoxins, amsacrine and anthracenediones are topoII inhibitors. This implies that cell lines which have an altered topoII will, in general, display crossresistance to all these drugs. The spectrum of cross-resist-

ance, therefore, does not include vinca-alkaloids, and in this it differs from the typical MDR phenotype. Moreover, there is not usually defective uptake in these selected cell lines. Antineoplastic drugs interact with topoisomerase by interference with the religation step of the reaction, which leads to a stable cleavable complex. Eventually, DNA strand breaks occur initiating a cascade of toxic events for the cells which culminate in cell death. Reduced levels, or mutations of topoisomerase genes have been identified as potentially responsible for drug resistance to topoisomerase inhibitors *in vitro* [11, 20–24].

Recently, Cole and associates described a multidrug resistance-associated protein (MRP) in a small cell lung cancer cell line which was selected for resistance to doxorubicin [25]. MRP belongs to the same ATP binding cassette superfamily of transporter proteins to which MDR1, MDR3 and many others also belong. Several cells have been described which have amplification of the *MRP* gene and overexpression of the protein in the absence of Pgp/MDR1 expression. MRP would also appear to have a function in transporting drugs through the membrane and out of the cell, leading to drug resistance.

The most recently described drug resistance-related protein is LRP (lung resistance protein). It was identified at the Free University in Amsterdam [26] as targeted by a monoclonal antibody (LRP56) which recognises several cell lines, where Pgp expression does not appear to have a role in determining the resistance. The recent cloning and sequencing of the gene do not suggest that LRP belongs to the same superfamily of transporters of MDR1 and MRP.

We have investigated the expression of Pgp and several other markers of MDR in samples obtained from 92 patients with primary breast cancer and 12 patients with metastatic disease using immunohisto/cytochemical assays and in selected samples using a sensitive and specific RNAse protection assay to assess MDR1 expression [27].

Pgp expression was observed significantly more often in the tumours of premenopausal than postmenopausal women ($P < 0.05$). Furthermore, there was a trend for Pgp-positive primary breast tumours to be associated with the presence of more than three positive axillary lymph nodes: 42% of Pgp-expressing primary breast cancers and 23% for Pgp-negative primary tumours ($P = 0.1$). With univariate analysis among the strongest prognostic factors for overall survival were the mitotic index, lymph node status, Pgp expression and, interestingly, the combination of Pgp-expressing tumour cells surrounded by Pgp-expressing stroma. Multivariate analysis for overall survival indicated the combination of Pgp^+ tumours plus Pgp^- stroma as the strongest prognostic factor ($P = 0.002$).

It is possible that Pgp expression could represent a marker of a more malignant phenotype and/or of drug resistance. In agreement with the former hypothesis, Pgp was, in fact, more frequently expressed in premenopausal breast cancer patients, and a trend was found in the correlation between expression in the primary tumour and axillary lymph node metastases. Futhermore, Pgp expression was found more often in metastatic than in primary breast cancer.

The combination of Pgp expression in tumour and desmoplastic stroma cells may identify a subgroup of very aggressive tumours. Stromal–epithelial communication can play an important role in these tumours [28]. Recently, tumour microvessel density has been reported to be an independent prognostic indicator in lymph node-negative breast cancer patients, as patients with tumours with a high microvessel density have a significantly higher risk of developing metastases [29].

Table 2. Frequency of expression of MDR-related genes

	Positive cases (%) Breast cancer			
Protein	Normal breast ($n = 6$)	Operable primary ($n = 20$)	Locally advanced ($n = 22$)	Metastatic ANT-resistant ($n = 10$)
Pgp	<25%	<25%	>75%	<25%
MRP	25–75%	>75%	>75%	ND
LRP	>75%	25–75%	25–75%	25–75%
TopoII-α	0	25–75%	ND	25–75%
TopoII-β	>75%	>75%	ND	>75%
TopoI	0	25–75%	ND	25–75%

ANT, anthracycline; ND, not done.

In another set of patients we performed a more extensive analysis of drug resistance-related genes, the tumour material originating from 60 patients who had been treated at various stages of breast cancer [30]. Normal breast tissue was collected from 6 patients who had undergone cosmetic surgery. Locally advanced breast cancer patients were treated with neoadjuvant chemotherapy consisting of high dose doxorubicin (90–100 mg/m^2) and cyclophosphamide (1 g/m^2) every 3 weeks, with GM-CSF support, followed by mastectomy.

Anthracycline-resistant patients undergoing an investigational trial with paclitaxel (3-h infusion of 250 mg/m^2 every 3 weeks, with G-CSF support) had biopsies taken from skin metastases prior to the first paclitaxel cycle, and again on progression. Anthracycline resistance was defined as progression on an anthracycline-containing regimen when at least two cycles had been administered. The expression of Pgp, topoI, topoII-α, topoII-β, MRP, and LRP56 were investigated in these tumour samples by immunohistochemistry and/or RNase protection assay [30].

By examining the differences in expression between the groups of patients and tissues analysed (normal breast, operable primary breast cancer, locally advanced breast cancer and metastatic anthracycline-resistant breast cancer), the preliminary results show that locally advanced breast carcinomas (T3–4) had significantly higher expression of Pgp than the primary operable breast cancers. This might indicate a poorer prognosis of the more advanced tumours, perhaps based on progressive selection of resistant clones.

In normal breast tissue, there was low expression of topoII-α, topoI, variable expression of Pgp/MDR1 and high expression of MRP and topoII-β. In primary operable breast cancer, there was low expression of Pgp/MDR1, approximately 25–75% of cells were positive for topoII-α and topoI, whereas the majority of cases were positive for MRP and LRP56 (Table 2). However, the levels of expression of MRP were, in general, only infrequently higher than those of GLC4, a relatively chemosensitive cell line.

To date, there has been no indication that single expression of the genes examined would be solely responsible for the sensitivity/resistance to chemotherapy in any of the groups analysed. Overall, the expression patterns of the assessed genes have failed to explain the therapy outcome of resistance to anthracyclines and taxoids in patients with advanced breast cancer. However, because the number of cases evaluated was relatively small, further investigation is ongoing.

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