EJC SUPPLEMENTS 7, NO. 1 (2009) 3–7

available at www.sciencedirect.com

Mechanisms and consequences of chemotherapy resistance in breast cancer

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ARTICLE INFO

Keywords:
Metastatic breast cancer
Chemotherapy
Resistance
MDR
Epothilones

ABSTRACT

Resistance to chemotherapy is a substantial clinical problem limiting the effectiveness of anticancer drug treatment. Resistance accounts for treatment failure in more than 90% of patients with metastatic disease. Overcoming mechanisms of resistance is crucial for the effective management of breast cancer, particularly once the disease has metastasised. However, approaches to reverse multi-drug resistance (MDR) have so far met with limited success. Targeted therapies are now well established in the clinic. Developmental agents with improved specificity for tumour cells also show promise. In addition, novel cytotoxics such as the epothilones, which have low susceptibility to some of the common types of drug resistance and have demonstrated activity in taxane-resistant breast cancer, also show promise. We are now in a new era for cancer therapeutics where there are increasing treatment options for oncology patients. There is, therefore, some optimism for the improvement in the management and survival of patients with metastatic breast cancer.

1. Introduction

Resistance to current chemotherapeutic agents accounts for the failure of treatment in more than 90% of patients with metastatic cancer,[1] the inevitable outcome being death. Drug resistance may be present prior to initial exposure to chemotherapy (intrinsic resistance), acquired over the course of treatment (extrinsic resistance) or inducible, in which cancer cells undergo genetic or epigenetic changes leading to chemoresistance.[2]

A number of mechanisms by which human breast cancer cells are, or become, resistant to chemotherapeutic agents have been described (Table 1; adapted from Michor et al. 2006[3]). These include exclusion or active export of drug from the cell, sequestration of drug within defined cellular compartments, activation of detoxifying enzymes and suppression of apoptosis. Additional factors such as DNA repair defects[4] and epigenetic regulation may also be involved in the development of drug resistance.[5]

Overcoming mechanisms of drug resistance is crucial for the effective management of breast cancer, particularly once the disease has metastasised. This paper reviews the mechanisms by which breast tumour cells develop drug resistance and explores approaches to overcome such mechanisms with novel agents, combination regimens and consideration for appropriate drug sequencing throughout the course of the disease to achieve maximal long-term disease control.

2. Drug resistance mechanisms in breast cancer

Chemoresistance affects a number of drug classes used in first-line therapy of breast cancer including the anthracyclines and taxanes (Table 1).

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1359-6349/ $ – see front matter

Table 1 – Mechanisms of chemotherapeutic drug resistance[a]

Type of resistance	Specific mechanism	Drug classes affected
Exclusion/containment	Sequestration	Anthracyclines Platinum Antifolates
	Increased drug efflux • ABC transporters e.g. P-glycoprotein • MDR proteins e.g. RLIP76	Anthracyclines Taxanes Platinum Vinca alkaloids Topoisomerase I/II inhibitors
	Decreased drug influx • Inactivating mutations or decreased expression of carrier molecules	Antifolates Nucleoside analogues
Drug target modification	Binding site modification	Taxanes Antifolates Topoisomerase inhibitors
Drug detoxification	Activation of detoxifying enzymes such as glutathione-S	Nucleoside analogues Anthracyclines Vinca alkaloids
Avoidance of tumour cell death	Blocked apoptotic signalling	Platinum Taxanes Anthracyclines
DNA repair mechanisms	Activation of DNA repair Deactivation of DNA repair (mis-match repair) due to gene silencing	Platinum

[a] Adapted from Michor et al. (2006).[3]

2.1. *Increased drug efflux from breast cancer cells*

Drugs such as anthracyclines and taxanes, which enter the cell by passive diffusion, may then be actively exported across the membrane leaflet via an ATP-dependent "flipase" process effected by ATP binding cassette (ABC) transporters including P-glycoprotein (P-gp) and multi-drug-resistance proteins (MRPs).[6] ABC transporters are found in all organisms and are usually specific for a single ligand while MRPs have a large flexible drug-binding site which confers activity against multiple drugs including anthracyclines and taxanes. These proteins may be overexpressed in tumour cells or following chemotherapy administration and can produce cross-resistance to other drug classes.[7]

ABC transporters actively transfer drug molecules from the cytoplasm and out across the cell membrane thereby preventing them from exerting their cytotoxic effects on microtubule dynamics and cell division. This mechanism is of particular relevance in the treatment of breast cancer as an estimated 40% of all breast tumours express P-gp.[8] Moreover, prior exposure to chemotherapy or hormonal therapy has been shown to increase the proportion of breast cancers expressing P-gp by 1.8-fold.[9] However, pre-chemotherapy P-gp expression showed no association with shorter progression-free survival (PFS) so the clinical relevance of this observation in terms of screening patients and treatment selection remains unclear.[9,10] MRP1 expression, on the other hand, has been associated both with shorter PFS and decreased overall survival (OS) in patients with MBC.[9] However, in spite of these interesting and careful studies, the relevance of ABC transporters in mediating MDR in the clinical setting remains unclear for metastatic breast cancer and, indeed, for other types of tumours. Moreover, the knowledge of these MDR mechanisms gained to date has not yet led to useful changes in clinical practice in terms of the management of drug-resistant patients.

2.2. *Drug target modification in breast cancer cells*

The efficacy with which target site modification can confer drug resistance is exemplified by the resistance typically acquired by human breast cancer cells towards the taxanes. These agents are involved in the stabilisation of tubulin polymers and act by inhibiting depolymerisation resulting in mitotic arrest and initiation of apoptosis. While drug efflux via P-gp is an important mechanism of taxane resistance, sensitivity to taxanes may also be affected by altered expression of microtubule-associated proteins, tubulin mutation, or altered expression of β-tubulin isotypes (Fig. 1).[11,12] These variations may include β-tubulin mutations, overexpression of βIII-tubulin, altered tubulin expression or alterations in the binding site. Overexpression of βIII-tubulin has been linked to both intrinsic and acquired taxane resistance in several cancer cell lines.[14,15]

2.3. *Accumulation of multiple resistance mechanisms*

Recent data have indicated that breast cancer cells can acquire multiple mechanisms of chemoresistance

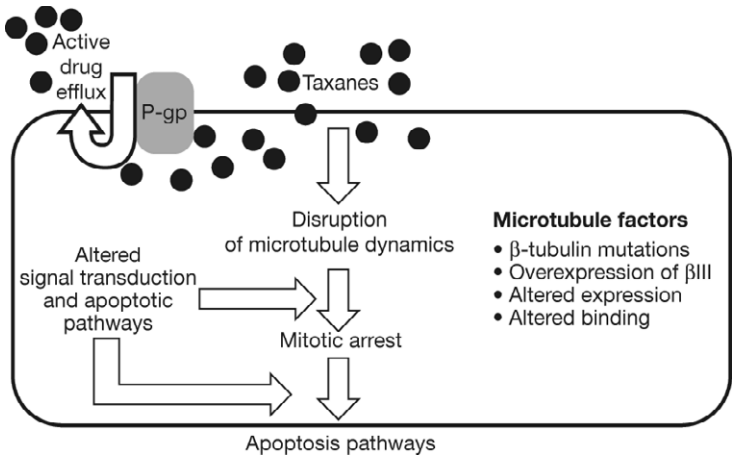

Fig. 1 – The multiple mechanisms of resistance to taxanes. Adapted from Coley (2008). [13]

against the taxane paclitaxel. [16] *In-vitro* studies demonstrated that the human breast cancer cell line MCF-7 can acquire resistance to paclitaxel over a number of weeks via exposure to low doses of the drug. During this period P-gp is gradually upregulated and caspases 7 and 9, which are involved in mitochondrial pathways to apoptosis, are expressed at reduced levels. These data suggest that paclitaxel resistance is multifactorial and does not arise solely through the upregulation of ABC transporters. [16]

3. Strategies for overcoming multi-drug resistance

A significant challenge in the management of breast cancer across the spectrum of the disease from its earliest stages through to metastatic disease is to overcome innate and emergent drug resistance. Understanding the ways in which breast cancer cells are, or become, resistant to current cytotoxics has opened up potential mechanisms to overcome such resistance.

3.1. *Inhibition of ABC transporter activity*

One potential mechanism might be through the inhibition of P-gp activity. However, as multiple transporters, each with differing specificities, may be responsible for resistance against a certain drug or class or drugs, multiple inhibitors may be required, making treatment regimens unfeasible. [6] Moreover, such an approach may result in inhibition of the normal and necessary function of P-gp with consequent unwanted side effects. [6] Finally, novel multi-drug transporters may arise by mutations that overcome the effect of targeted inhibitors or by altering ABC transporter binding activity. [6] Although several P-gp inhibitors have been evaluated the results have been somewhat disappointing. [17,18]

3.2. *Disruption of ABC transporter binding*

An alternative approach might be to alter the structure of the target drug in order to disrupt binding with the partner ABC transporter. However, unlike enzymes with high specificity, small modifications in drug structure are not generally sufficient to modify resistance to any relevant extent and large modifications may lead to decreased cytotoxicity. [6]

3.3. *Novel agents able to overcome multi-drug resistance mechanisms*

Currently, the greatest hope of clinically significant advances in this respect lies in the discovery and development of new agents that are not susceptible to multi-drug resistance mechanisms. A variety of such novel antineoplastic agents are currently in development, including poly(ADP-ribose) polymerase (PARP) inhibitors for use in BRCA-associated breast cancer, novel targeted therapies such as lapatinib, epigenetic reversal agents such as 5-azacytidine and novel microtubule-destabilising agents.

The epothilones have recently emerged as a promising approach to overcoming chemoresistance in patients resistant to taxanes. Epothilone-B derivatives exert their cytotoxic effect by binding to tubulin, stabilising microtubule dynamics and thereby inhibiting cell division. Ixabepilone is the most developmentally advanced of the epothilones and the first to be approved in the USA for use in the management of metastatic or locally advanced breast cancer after resistance to an anthracycline and a taxane. Ixabepilone is distinct from the taxanes and is less susceptible to known resistance mechanisms. Indeed, ixabepilone retains activity in cell lines exhibiting a variety of resistance mechanisms against paclitaxel including increased drug efflux, tubulin alteration and overexpression of βIII-tubulin (Fig. 2). [19,20] Ixabepilone is a poor substrate for P-gp and has demonstrated low

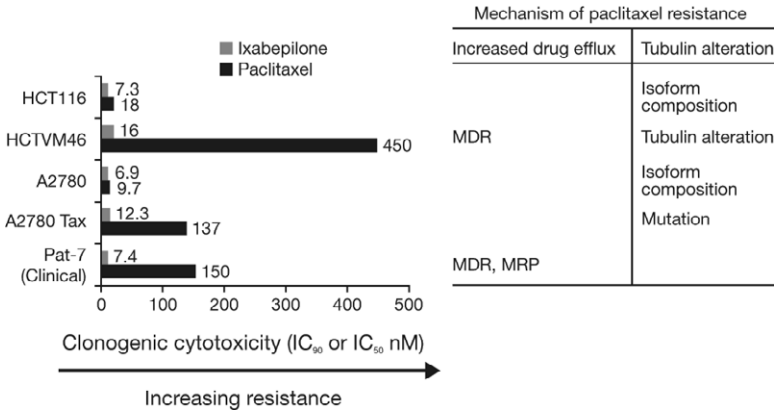

Fig. 2 – Epothilone (ixabepilone) activity in cell lines resistant to taxanes via increased drug efflux and tubulin alteration. [20]

susceptibility to MRP1. [15] Moreover, ixabepilone remains active in taxane-resistant breast cancer xenografts. [20]

With this knowledge in hand, it may be that in future, screening of patients for factors known to induce or confer resistance, including MRP expression and βIII-tubulin overexpression, may help to guide selection of the most effective treatment options.

4. Conclusions

Resistance to chemotherapeutic agents is a significant issue in the management of patients with breast cancer, particularly those with metastatic disease whose treatment options are often limited. Multiple mechanisms, including active drug efflux and binding site modifications, are implicated in resistance of breast cancer cells to the most widely used chemotherapeutics, the anthracylines and the taxanes. Indeed, it now appears that individual cell lines may accumulate multiple mechanisms of resistance against individual agents. Consequently, efforts to regain activity of agents through, for example, inhibition of ABC transporters, may be limited or short-lived. For breast cancer patients who are, or become, resistant to current cytotoxic agents, novel agents with less susceptibility to some of the important known multi-drug resistance mechanisms are needed. One such group of novel agents are the epothilones, including ixabepilone, the first epothilone to be approved for use in drug-resistant patients with metastatic breast cancer.

Acknowledgements: Medical writing and editorial assistance, provided by Gardiner-Caldwell U.S., was supported by Bristol-Myers Squibb.

Competing interests: Helen Coley received speaker's honoraria from Bristol-Myers Squibb.

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