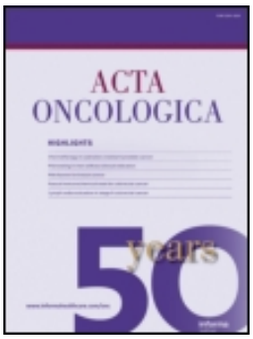

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CHEMOTHERAPY RESISTANCE MECHANISMS

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Resistance to chemotherapy is a major problem in oncology. The drug resistance in breast cancer shares most traits with other solid tumours. As a result of studies of cell lines and animal tumours a number of drug resistance factors have been identified but only limited data from human tumours are available.

We consider studies concerning drug resistance in breast cancer to be of particular interest. During the last few decades data have accumulated, indicating that adjuvant chemotherapy of breast cancer patients may prolong survival (1). Recent studies have also shown that high dose chemotherapy may improve survival in patients with advanced breast cancer (2). Most probably, the therapy might be improved if techniques for detection of microscopic disease and prediction of chemotherapy response were available.

Several factors contribute to drug resistance. Physiological resistance to chemotherapy implies the role of tumour blood flow, the pH in the tumour tissue as well as oxygenation in tumour metabolism. Biological resistance refers to factors like tumour cell heterogeneity, as well as its metastatic phenotype. Traditionally, the term 'drug resistance' refers to biochemical resistance, which means that certain molecules are responsible for the drug resistance. This paper summarises data on some biochemical resistance factors which also have a potential role in cytostatic treatment of breast cancer.

Multidrug resistance

MDR1

The multidrug resistance (MDR) phenotype is well characterised in experimental systems (3). There is a decreased accumulation of cytotoxic drugs; there are changes in the activity of expression of certain cellular proteins; the phenotype also includes changes in the cellular physiology affecting the structure of the plasma membrane; the intracellular transport of membranes, as well as the lysosomal structure and function are often changed.

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Several proteins are often overexpressed in cells with the MDR phenotype: the p170 glycoprotein, encoded by the MDR1 gene (4, 5); the multidrug resistance protein (MRP); glutathione transferases; and metallothionein. Topoisomerase II is often underexpressed in cells with this phenotype.

The MDR1 protein product is a 170 kD transmembrane glycoprotein involved in transporting molecules out from the cell. Biological cytostatics such as anthracyclines are recognised by the p170 glycoprotein. Transfection of cells with the gene coding for the p170 glycoprotein confers an MDR phenotype (6, 7). There is also a second MDR gene having 77% sequence identity with the MDR1 gene, but transfection of this gene did not affect drug resistance.

In experimental systems the resistance caused by the MDR1 gene can be reversed. Cells can be converted to anthracycline sensitivity by calcium antagonists like verapamil (8). Cyclosporin PSC-833 (9) as well as quinidine (10) can reverse MDR1 resistance. Drugs which interfere with the MDR1 efflux pump have been studied in minor series of patients with breast cancer receiving chemotherapy. Results, however, are not conclusive. A prospective phase III study of quinidine showed no in vivo modulation of MDR (11).

In tumour tissue from untreated patients with breast cancer the MDR1 expression is low. In the summary of three studies including 317 patients only 3% of the subjects had tumours which expressed the p170 glycoprotein. There are, however, indications that patients treated with anthracycline-containing regimens may develop tumours with increased expression of the p170 glycoprotein (12–14). The MDR1 gene product is considered a clinically interesting drug resistance factor in breast cancer. Its potential role should be further studied in prospective clinical trials.

Glutathione transferases

Glutathione conjugation is an established drug resistance factor in many experimental systems. The cell is protected against a number of electrophilic molecules by this inactivation mechanism. Small molecules may react spontaneously with glutathione. For molecules of larger sizes the reaction may be mediated by glutathione transferases (GSTs). There are four classes of GSTs: Alpha,

Mu, Pi and Theta. The cytosolic GSTs are dimeric proteins. In vitro, significant catalytic activity for GSTs A1-1 (class Alpha) with chlorambucil has been shown (15). Catalytic activity has also been demonstrated for GSTM3-3 (class Mu) with BCNU (16). The expression and activity of GST Alpha has been correlated to the resistance against nitrogen mustard derivatives in several experimental systems. A variable expression and activity have been shown for several human tumours, including breast cancer (17). The potential role of GSTs as drug resistance factors in cytostatic treatment of human tumours remains to be shown. A current hypothesis is that tumour cells may express mutated forms of GSTs with increased catalytic activities.

An interesting observation is that ras-transformed human mammary epithelial cells had increased resistance against cisplatinum (18). The resistance is correlated to a decreased level of DNA damage. The transcription factor AP-1 contains the protein products of the fos and jun oncogenes. A consequence of ras activation is increased expression of AP-1. In experimental systems this factor increases the expression of GSTs. Thus, ras activation may increase drug resistance, at least partly, by influencing the expression of GSTs. In melanoma metastases, co-expression of N-ras and GST Pi has been shown (19).

The multidrug resistance protein (MRP)

Multidrug resistance protein (MRP) is a 180–195 kD membrane glycoprotein (20). Overexpression of this molecule increases resistance to several naturally occurring cytotoxic drugs. MRP binds to glutathione conjugates and the formed complex of molecules can be transported out from the cell (21). Depletion of cellular glutathione content by treating the cells with buthionine sulphoximine, an inhibitor of the glutathione synthesis, increases the intracellular accumulation of anthracyclines (22). Thus, the cellular glutathione levels may regulate the transport of drugs by MRP (23), which is a potential drug resistance factor in breast cancer.

Metallothionein

Metallothionein plays a role in detoxification and confers resistance to ionizing radiation and alkylating agents. It has been shown that the expression of metallothionein is correlated to the prognosis in breast cancer (24). Whether it is involved in drug resistance in breast cancer remains to be shown.

Aldehyde dehydrogenases

The aldehyde dehydrogenases are a family of isoenzymes which may participate in the intracellular detoxification of cyclophosphamide (25, 26). The enzyme catalyses the conversion of aldophosphamide to carboxyphosphamide, an inactive compound. Thus, increased aldehyde dehydrogenase expression is a potential resistance factor.

Antimetabolites

The most frequently used antimetabolites in the treatment of breast cancer are methotrexate and 5-fluorouracil. The cellular uptake of methothrexate is regulated by active transport over the cell membrane. The intracellular drug concentration is also dependent on an active efflux pump. The intracellular concentration determines the degree of inhibition of the dihydrofolate reductase, the target for methotrexate. The expression of this enzyme is a resistance factor.

5-fluorouracil is converted by the cell to the phosphorylated compounds 5-FdUMP and 5-FTP. FdUMP binds to thymidylate synthetase, which is the rate-limiting enzyme in thymidine triphosphate synthesis. DNA replication is inhibited, which is the most important mechanism. Thus, expression of thymidylate synthetase may modulate the sensitivity to 5-fluorouracil. Further, metabolites of 5-fluorouracil are substrates for both DNA and RNA polymerases, with incorporation to both DNA and RNA as consequences.

It has been discussed whether amplification of the genes for dihydrofolate reductase and thymidylate synthetase is a resistance mechanism, as it has been shown for animal tumour cell lines. So far, most studies on human tumour cells indicate that increased expression may be a resistance factor, but not synonymous with gene amplification. Although conclusive clinical studies on breast cancer are lacking, thymidylate synthetase is an interesting potential drug resistance factor.

Topoisomerases

The topoisomerases regulate the topological state of DNA during replication and transcription. Topoisomerase II is a target for the anthracyclines, which bind to the enzyme and stabilise the topoisomerase II-DNA intermediate. An increased expression of topoisomerase II is accompanied by increased sensitivity to anthracyclines. There are few clinical studies so far on breast cancer but indications of a relationship between the mRNA expression of topoisomerase II and response to anthracyclines have been reported (27). It has also been shown that the gene for topoisomerase II can be amplified in primary breast tumours (28).

DNA repair

O6-alkylguanine-DNA alkyltransferase (O6-AT)

O6-AT is a DNA repair protein which eliminates methyl- and ethyl groups from the O6 atom of guanine in

DNA (29). Methyl- or ethyl groups are transferred to a cystein acceptor residue in the O6-AT protein which is thereby irreversibly inactivated. De novo synthesis of the protein is required for repair to continue. There are a number of in vitro studies linking the expression and activity of O6-AT to the toxic effects of chloroethyl nitrosoureas and triazenes. A relationship between the sensitivity to chloroethyl nitrosoureas and activity of O6-AT has also been shown for human malignant gliomas (30). About 10% of tumour samples from patients with different malignant tumour types including breast cancer (31) show low or no activity of the enzyme. A current hypothesis is that tumours with low or no activity of O6-AT are those which respond to chloroethyl nitroso-ureas and triazenes. It is particularly interesting that there are inhibitors against the O6-AT. Benzylguanine, which is an effective inhibitor of the enzyme activity in experimental systems may be a candidate for reversing resistance in patients. O6-AT should be studied further in breast cancer.

Nucleotide excision repair

There are two forms of nucleotide excision repair: transcription-coupled repair, which takes place on active transcribed genes and global genome repair. In eucaryotic cells about 30 gene products are thought to be involved in nucleotide excision repair. There are links between nucleotide excision repair and cell cycle regulation, recombination processes, DNA replication, chromatine structure and transcription (32).

UV-sensitive Chinese hamster mutant cells have been isolated. Excision repair cross-complementing (ERCC) genes have been cloned from these cells and the cloned genes have been used to characterise nucleotide excision repair in human cells. Some of the mutants (1, 4 and 11) show an extreme sensitivity to cross-linking agents.

The nucleotide excision repair involves six steps: search for the DNA damage, damage recognition, lesion demarcation, dual incision, release of damaged oligonucleotides and, finally, gap filling DNA synthesis.

Repair of UV-induced DNA damage has been thoroughly studied. It has also been shown that DNA damage induced by alkylating agents and platinum compounds may act as a substrate for nucleotide excision repair. In a number of studies dealing with cell lines the DNA repair process has been blocked and, as a consequence, increased sensitivity to DNA-damaging agents has been registered. More studies are required to consider the potential role of nucleotide excision repair in the treatment of clinical tumours.

Apoptosis and p53

Apoptosis occurs naturally in human tumours. A high apoptotic activity will result in retarded tumour growth. It has been shown that apoptosis is increased after UV-radiation, ionizing radiation, chemotherapy, heating and hormonal treatment (33–35). Apoptosis is regulated by both protooncogenes and tumour suppressor genes. It may be increased by C-myc and decreased by Bcl2 and is also regulated by the p53 tumour suppressor gene. In p53 knock-out mice, apoptosis induced by DNA damage is decreased. Studies on cell lines give experimental evidence that altered p53 function results in an increased resistance to different types of DNA damaging agents (34).

In a summary of 11 studies including 603 patients with breast cancer 143 (24%) showed alterations in the p53 gene (36). The mdm-2 gene product inactivates p53. Amplification or altered expression of this gene may be an additional mechanism by which p53 is inactivated. There are observations indicating that alterations of the p53 gene product may influence prognosis in breast cancer (37). It has been suggested that resistance to treatment may be modulated by mutations in the p53 gene (38, 39).

Knowledge of drug resistance factors: clinical implications

The clinical use of methods to predict chemotherapy response has so far been limited. The problems relating to techniques based on short-term culture of tumour cells are pronounced. The identification of molecules responsible for drug resistance offers new possibilities for selecting patients with sensitive tumours. Strategies similar to those employed in endocrine therapy should be developed for chemotherapy. High dose chemotherapy adjusted to the expression of drug resistance factors should increase the probability of cure, both for patients with advanced breast cancer and for those with microscopic disease.

In order to evaluate the contribution of different drug resistance factors, several factors should be simultaneously estimated in the same tumour, analogously with the way prognostic factors are evaluated. Owing to the development of highly sensitive molecular genetic techniques such as reverse transcriptase polymerase chain reaction, fine needle aspiration biopsies can be used to estimate the expression of several drug resistance factors in the same tumour sample. The heterogeneity of the cell population may be determined by immunohistochemistry.

Knowledge of drug resistance factors can also be used to circumvent drug resistance. The MDR phenotype may be reversed by several strategies in experimental systems. In several clinical studies results have been negative, mainly due to toxicity by the reversing agent. Verapamil may reverse MDR1 resistance. Cyclosporin PSC 833 is an effective MDR1 reversing substance. Furthermore, the anti-MDR1-ribozyme may inactivate the MDR1 protein. There are several ways to interfere with the DNA repair processes. The O6-AT activity can be modulated by benzylguanine. Phase I studies are ongoing to evaluate toxicity induced by this inhibitor. Buthionine sulphoximine is un-

der clinical evaluation in order to study its clinical effects concerning glutathione depletion. The regulation of apoptotic cell death may also be manipulated for cytostatic regimens causing cell death by apoptosis. In all probability, the rapid development of knowledge concerning cell cycle regulation will give a deeper insight into how chemotherapy may interfere with this regulation and open possibilities to modify strategic factors aimed at increasing the cytotoxic effects.

Most likely, a pronounced effect on the probability of curing patients will be obtained if prediction of chemotherapy response is combined with techniques for improved detection of minimal disease. Reverse transcriptase polymerase chain reaction techniques have been used for the detection of mammary carcinoma cells in lymph nodes, bone marrow and the blood (40, 41). An alternative technique is flow cytometry after labelling tumour cells with monoclonal antibodies (42). Breast cancer is considered an interesting tumour for the development of new strategies based on knowledge of drug resistance factors and minimal disease.

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