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Prediction of the response to chemotherapy in ovarian cancers

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Ovarian cancer represents the fifth most frequent cause of death as a result of malignant processes after cancers of the breast, large intestine, lung and stomach. Owing to the localisation of ovarian cancer, approximately 75% of cases are diagnosed at the III and IV stages of advancement according to FIGO. Because of the advanced stage of the disease surgery has to be followed by chemotherapy in most cases of ovarian cancer and therefore resistance to cytostatic drugs represents a major clinical problem. The potential to predict the response to therapy with the use of cytostatic drugs would enable the most effective drugs to be applied in individual cases, thus improving the efficiency of the treatment and restricting the development of resistance to cytostatic drugs. In the present paper the progress made so far in the prediction of the clinical course of ovarian cancer is reviewed. The significance of the expression of the ATP-binding cassette (ABC) transporters is described, including P-glycoprotein and MRP2, the principal representatives of the protein group. The importance of disturbed control of apoptosis and the overexpression of HER-2 and topoisomerase 1A are also discussed. Two sections are devoted to the most recent studies in the biology of ovarian cancer, pangenomic studies on gene expression using DNA microarrays and aberrations of DNA methylation.

Key words: ovarian cancer, chemotherapy, prediction, multidrug resistance

INTRODUCTION

Owing to the localisation of ovarian cancer, approximately 75% of cases are diagnosed at the stages III and IV of advancement according to FIGO. In such advanced cases only about 20% of patients survive for 5 years. Because of the advanced stage of the disease surgery has to be followed by chemotherapy in most cases of ovarian cancer. Despite the introduction of novel chemotherapy regimens, the five-year survival rate of patients at all clinical stages has not risen beyond 40% in the last 20 years [3, 7]. Resistance of ovarian cancer cells to cytostatic drugs represents the principal cause of therapeutic failure and, consequently, the principal cause of death in cases of ovarian cancer.

ATP-BINDING CASSETTE (ABC) TRANSPORTERS

The phenomenon of multidrug resistance (MDR) was described for the first time at the end of the 1980s [13]. At the time it was demonstrated in *in vitro* studies that the development of resistance to one antineoplastic agent resulted in a cross-resistance to several other cytostatic drugs which were not inter-related. The principal causes of MDR in cancer cells are proteins from the group of

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ATP-binding cassette (ABC) transporters, the membranous ATP-dependent pumps, including P-glycoprotein, MRPs and BCRP proteins [13, 14].

P-glycoprotein (P-gp)

P-glycoprotein (P-gp) is a member of the family of ABC-transporter proteins. P-gp is an energy-dependent pump, which in physiological conditions is manifested in the cell membranes of, for example, hepatocytes and the cells of renal tubules and is responsible for the transportation of various substances out of the cell. In tumours P-gp actively eliminates cytostatic drugs, and overexpression of P-gp is linked to resistance to cytostatic drugs [13, 14, 62]. P-gp is composed of two parts of a similar structure, each containing a hydrophobic and a hydrophilic part. The hydrophobic part encompasses three loops, which span the cell membrane at six sites. The six transmembrane domains form a canal through which substances can cross the cell membrane. The hydrophilic part, located on the cytosolic aspect of the membrane, contains potential ATP nucleotide-binding sites. Energy originating from ATP hydrolysis is utilised for the transportation of substances across cell membranes [13, 14].

It has been demonstrated that P-gp actively eliminates from the cell cytostatic drugs of various therapeutic groups (Table 1) [13, 14]. The silencing of the *MDR1* gene coding for P-gp makes tumour cells sensitive to cytostatic drugs [28]. The unfavourable significance of P-gp expression has been documented in numerous tumours treated by chemotherapy (Table 1), including ovarian cancers [13, 32, 47].

An interesting trend in P-gp-linked studies was started by the study of Patel et al. [35], who described an experiment in which cell lines of rat kidney mesangium were transfected with the gene coding for cyclooxygenase-2 (COX-2). The transfection with COX-2 was found to be followed by a significant increase in P-gp expression. In our studies, performed on sections from ovarian cancers [47] or breast cancers [51], a strong positive correlation was detected between expressions of COX-2 and P-gp. The reports may carry significant clinical implications. The authors suggest that the application of economical and well tolerated drugs which inhibit the activity of COX-2, such as aspirin or non-steroid anti-inflammatory drugs, may markedly affect the efficacy of tumour chemotherapy and also the expression of P-gp.

MRPs

MRP proteins (MDR-related proteins) form a family of proteins, including MRP1, MRP2, MRP3, MRP4, MRP5, MRP6, MRP7, MRP8 and MRP9 proteins. In the phenomenon of resistance to cytostatic drugs the most important role seems to be played by MRP1-MRP4 proteins [13, 14].

MRP1 (glutathione and glucuronate conjugate pump and resistance factor for anthracyclines, epipodophyllotoxins, vinca alkaloids and campothecins) consists of 1531 amino acids. Its sequence manifests a 15% homology with that of P-gp. The protein is involved in the transportation of organic anions. Overexpression of MRP1 is linked to augmented activity of the ATP-dependent transportation of endogenous glutathione, glucuronate or sulphate

Table 1. Tissue localisation of selected ATP-binding cassette (ABC) transporters, their function and the significance of their expression in tumour cells

Common name	Tissue	Chemotherapy substrates	Expression described in tumours
PGP/MDR1	Intestine, liver, kidney, placenta, blood-brain barrier	Doxorubicin, daunorubicin, vincristine, vinblastine, actinomycin-D, paclitaxel, docetaxel, etoposide, teniposide, bisantrene	Ovarian, breast, lung, adrenal, kidney, liver, colon, pancreas, lymphoma, neuroblastoma, leukaemia
MRP1	All tissues	Doxorubicin, epirubicin, etoposide, vincristine, methotrexate	Ovarian, oesophagus, gastric, thyroid gland, lung, lymphomas, neuroblastoma, leukaemia
MRP2	Liver, kidney, intestine	Cisplatin, methotrexate, etoposide, doxorubicin, vincristine, doxorubicin	Ovarian, colon, gastric, kidney, lung, leukaemia
MRP3	Pancreas, kidney, intestine, liver, adrenal glands	Etoposide, teniposide, methotrexate, cisplatin, vincristine, doxorubicin	Ovarian, kidney, lung, colon
MRP4	Prostate, testis, ovary, intestine, pancreas, lung	Methotrexate, thiopurines	Leukemia
BCRP	Brain, monocytes	Cisplatin, doxorubicin, daunorubicin, etoposid, epirubicin, mitoxantrone, topotecan, SN-38	Ovarian, colon, liver, lung, breast, placenta, leukaemia

conjugates [13, 14]. The protein is present in the muscles, lungs, spleen, urinary bladder, gallbladder and the suprarenal cortex. In the case of tumours the activity results in a lowered intracellular accumulation of cytostatic drugs, leading to resistance to these drugs (Table 1). Expression of the protein has been demonstrated in numerous tumours (Table 1) [13, 14]. In the case of ovarian cancers some reports have described expression of MRP1. Yakirevich et al. [60] failed to demonstrate a relationship between MRP1 expression and the survival of ovarian cancer patients. They documented a positive correlation between expression of MRP1 and the expression of other proteins linked to resistance to cytostatic drugs, such as p53 and BCL-2. Arts et al. [2] were also unable to show a relationship between expression of MRP1 and overall survival time, progression-free time and clinical response. On the other hand, they documented a positive correlation between expression of MRP1 and expressions of MRP2 and P-gp. Similarly, Katsaros et al. [25] detected a positive correlation between expressions of MRP1 on the one hand and P-gp and MRP2 on the other but found no relationship with the clinical response to chemotherapy.

MRP2 (canalicular efflux pump for amphipathic anions and resistance factor for anticancer agents), also termed cMOAT (canalicular multispecific organic anion transporter) is responsible for bilirubin glucuronate transportation from hepatocyte to biliary canaliculi. Mutation of the gene coding for MRP2 is linked to pathogenesis of Dubin-Johnson's syndrome. In physiological conditions the protein is manifest mainly in the liver, kidneys, intestines and central nervous system, in which it participates in the formation of the blood/brain barrier. Expression of the protein has been reported in numerous tumours (Table 1). Its activity is mainly linked to resistance to cisplatin (Table 1) and it should thus play a significant role in predicting the clinical response to chemotherapy in ovarian cancer [13, 14, 27, 30, 36, 52]. Studies on the relationship between the expression of MRP2 at the level of mRNA in ovarian cancers and clinical data have demonstrated no prognostic or predictive value in estimations of MRP2 expression [32]. Gumiński et al. [15] showed that immunohistochemical detection of MRP2 expression in ovarian cancer demonstrated a variable intracellular localisation of the protein. The authors detected no relationship between expression of MRP2 and the clinical data of the patients studied. We have documented that MRP2 may be present not only in plasma membrane, but also in the nuclear envelope of ovarian cancer cells (Fig. 1A, B, C) [50]. Ovarian cancer cases with nuclear MRP2 expression demonstrated a significantly shorter overall survival time. Silencing of the MRP2 coding gene using a multiribozyme resulted in a decreased expression of mRNA and nuclear expression of the protein (Fig. 1D) [28].

Expression of other MRP proteins in ovarian cancers has been poorly recognised or has not hitherto been studied.

BCRP

Breast cancer resistance protein (BCRP) belongs to subfamily G of the ABC transporters. Expression of BCRP has been described in several tumours (Table 1). Overexpression of BCRP results in resistance to a broad range of antineoplastic drugs [1, 40, 61]. Jia et al. [24] demonstrated that BCRP is responsible for the resistance of ovarian cancer cells to topotecan.

APOPTOSIS

Platinum analogues represent the drugs most frequently applied in the therapy of ovarian cancer. The mechanism of action of cisplatin and of other platinum analogues involves binding drug molecules to DNA (the formation of so-called DNA adducts). Cells may react to the formation of such adducts in various ways, depending upon the efficacy of their regulatory systems, proliferative activity and the number of adducts formed. One of the possible cellular reactions involves DNA repair and the other mobilisation of the process of cell apoptosis. The efficiency of a therapy incorporating platinum analogues thus reflects the efficacy of the mechanisms which control apoptosis. The best recognised apoptosis regulators of predictive value in ovarian cancers are p53, p21, p27 and BCL-2 [42, 55, 57].

p53

The most frequent genetic alteration in ovarian cancers involves mutations of the *P53* gene. *P53* is a suppressor gene and is localised on chromosome 17p13.1. p53 protein is a transcription factor which may induce or inhibit expression of several genes, including *MDM2, Bax, BCL-2, Fas.* In this way, p53 controls functions important for cell life: proliferation, apoptosis and DNA repair. If a cell contains mutated p53, the process of switching on apoptosis becomes less probable: tumour cells with mutated p53 continue to proliferate despite numerous lesions to DNA, induced by cytostatic drugs [26].

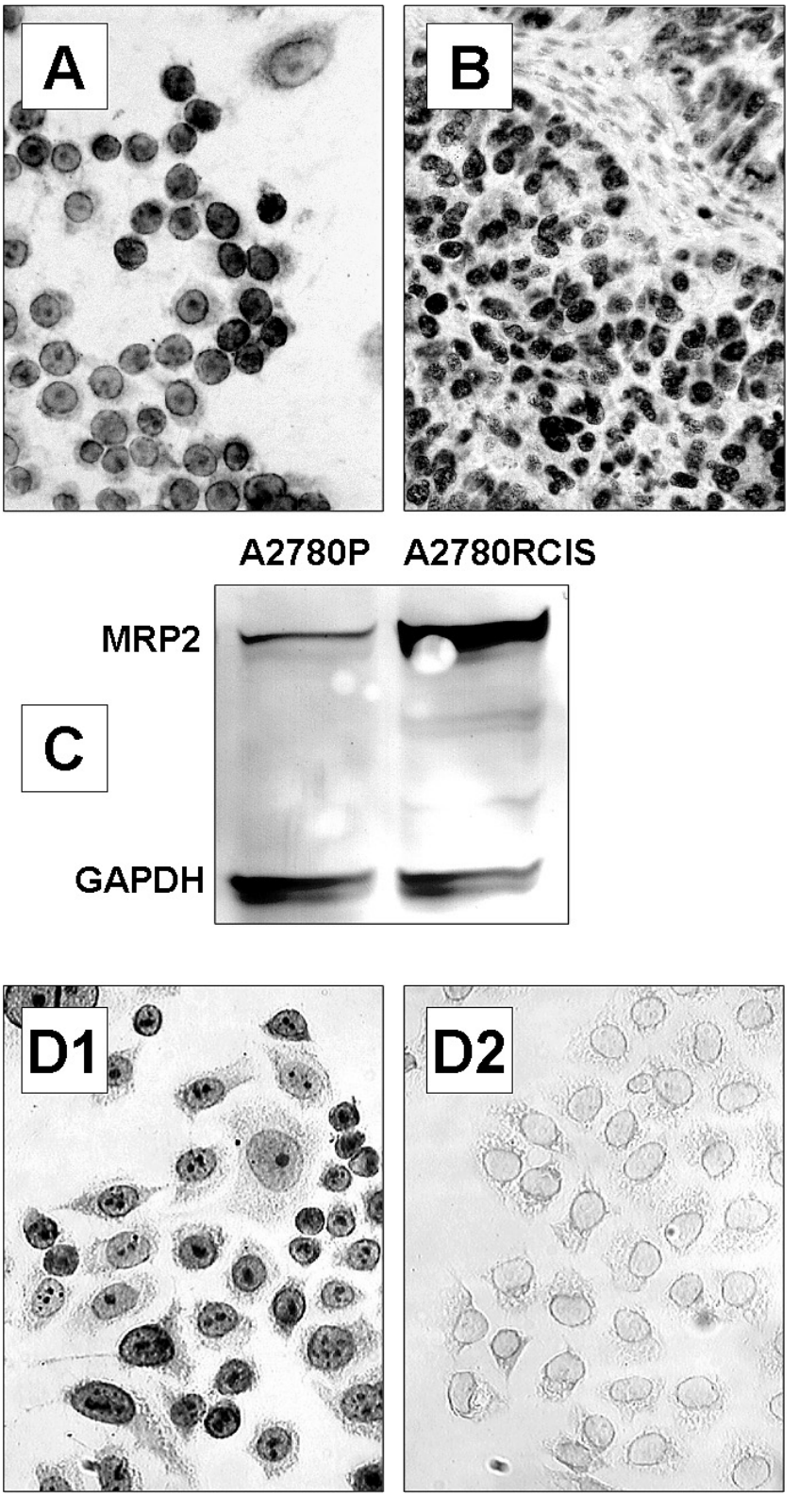

Figure 1. Immunohistochemical localisation of MRP2 expression in the nuclear membrane of: **A.** Cisplatin-resistant A2780RCIS ovarian carcinoma cell line; **B.** Ovarian carcinoma specimen; **C.** Western-blot analysis of MRP2 expression in the nuclear fraction of proteins in the cisplatin-sensitive A2780P ovarian carcinoma cell line and cisplatin-resistant A2780RCIS ovarian carcinoma cell line (GAPDH control, note stronger reaction intensity in cisplatin-resistant A2780RCIS cells); **D.** MRP2 expression in the nuclear membrane of: **D1.** Cisplatin-resistant A2780RCIS ovarian carcinoma cell line, **D2.** No reaction in cisplatin-resistant A2780RCIS ovarian carcinoma cell line transfected with multiribozyme directed against MRP2, P-gp and BCRP mRNA [32].

Mutations of the *P53* gene not only play a significant role in the pathogenesis of ovarian cancers but also in the biology. Tumours with overexpression of the p53 protein are less differentiated and manifest higher proliferative potential and a more aggressive clinical course [29, 37]. In *in vitro* studies cells of lymphoma, ovarian cancer and lung cancer with a mutated *P53* manifest a much lower sensitivity to cisplatin than do cells carrying no such mutation. Transfection of the mutated cells with the normal *P53* gene increased their sensitivity to cisplatin [11]. Numerous studies have demonstrated the unfavourable prognostic and predictive significance of p53 overexpression in ovarian cancers related to their response to treatment with cisplatin [12, 19, 20]. It is beyond doubt that p53 is one of the most important molecular prognostic and predictive indices in ovarian cancers. Immunocytochemical estimation of p53 protein expression seems to carry the highest prognostic and predictive value.

Proteins of the BCL-2 family

One of the earliest processes to develop in apoptosis involves release of proteins such as apoptosis inducing factor (AIF) and cytochrome C from the intermembranous space of mitochondria to the cytoplasm. The release of cytochrome C from the mitochondria remains under the strict control of proteins from the BCL-2 family. Some of the proteins, such as BCL-2, BCL-x_L, MCL-1 and BCL-W, prevent apoptosis, while others, such as BAX, BCL-x_S, BAK, BAD, BIK and BID, stimulate the process [6, 26, 55].

Augmented expression of proteins of the BCL-2 family, which inhibit apoptosis, represents an unfavourable predictive factor in therapy with platinum analogues. Many authors have shown the unfavourable predictive value of BCL-2 expression in ovarian cancers [42]. Verkis et al. [54] performed a statistical analysis of the generally available data on expression of 1416 genes in 60 cell lines (http://dtp.nci.nih.gov) and of their sensitivity to four distinct platinum analogues: cisplatin, carboplatin, oxaliplatin and tetraplatin. The studies demonstrated that resistance to cisplatin and carboplatin was paralleled to a high degree by overexpression of BCL-x_L. Williams et al. [58] described BCL-x_L expression in biopsies originating from 28 cases of ovarian cancer. The authors showed that overexpression of BCL-x_L was typical for patients with a shorter relapse-free survival. The results, however, require confirmation on more extensive material.

TRAPPING OF DRUGS IN CELLS

Numerous investigations have demonstrated that cytostatic drugs may be bound to proteins or glutathione in neoplastic cells. An example of such binding is provided by the binding of platinum analogues to metallothioneins.

Metallothioneins (MTs) are low molecular weight proteins (6–7 kDa) whose chains are composed of 61 or 62 amino acids. Typically, they contain multiple cysteine residues and few aromatic amino acids. MTs are thought to mediate several functions, including control of the levels of indispensable trace elements (such as zinc and copper), alleviation of the toxic effects of cadmium and mercury and protection of cells from oxidative stress [17]. Numerous reports have been published on MT expression in ovarian cancers [33, 46, 48, 59]. Opinions diverge on the relationship between MT expression and clinical course in ovarian cancer patients treated with various chemotherapeutic protocols based on platinum analogues.

Another example of the intracellular binding of drugs involves their conjugation with glutathione. The reaction is catalysed by glutathione transferases.

Glutathione S-transferases (GSTs) catalyse conjugation of reduced glutathione with a variety of electrophilic xenobiotics and also play a role in the degradation of lipid peroxidation products [5]. The resulting glutathione adducts have increased solubility and can then either be excreted or further metabolised.

The GST-pi isoform is best recognised and manifests a close relationship with resistance to cytostatic drugs. A relationship has been suggested between expression of and resistance to cisplatin, adriamycin, cyclophosphamide and etoposide [5]. However, the data on relations between expression of GST-pi and clinical response to cisplatin therapy are divergent: in several studies expression of GST-pi has been noted to correlate with a less favourable response to cisplatin [8, 41, 48], whereas no such relationship can be detected in other investigations [22].

HER-2

Human epidermal growth factor receptor (HER-2) belongs to the group of type I tyrosine kinase receptors for the human epidermal growth factor. The *HER-2* gene was found to represent a proto-oncogene. Amplification of the *HER-2* gene plays a significant role in the process of neoplastic transformation [43]. Amplification and/or overexpression of *HER-2* develops in 10–50% of cases of ovarian cancer and is linked

to a more aggressive clinical course of the disease. A relationship has also been demonstrated between HER-2 overexpression and resistance to cisplatin. In *in vitro* studies tumour cells transfected with the *HER-2* gene became resistant to cisplatin. Application of emodin, a tyrosine kinase inhibitor, and of antibodies specific for HER-2 was also found to augment the sensitivity of cells to cisplatin. Chemotherapy with cisplatin and cyclophosphamide was demonstrated to extend significantly the survival of patients with ovarian cancer who manifested a low expression of HER-2 but failed to exert such an effect in cases with overexpression of the protein [42, 55, 57].

TOPOISOMERASE 1A

Topoisomerases (TOP) are nuclear enzymes which transiently break und unwind DNA in the process of DNA replication and transcription [56]. Augmented expression of TOP 1A has been described in various types of tumours, including ovarian cancers [21, 34]. The protein provides a target for the group of anti-neoplastic drugs termed campothecins (topotecan and irrotecan), frequently used in ovarian cancers [56]. Expression of TOP 1A has been documented as a significant predictive index in campothecin-based therapy [39]. We have shown that ovarian cancer cases with augmented expression of TOP 1A demonstrated a significantly shorter overall survival time [49]. It should be noted that topotecan has not been used in the group of patients studied. The observations indicate that in cases with augmented expression of TOP 1A application of topotecan should be considered, as it might favourably change the prognosis in this group of patients.

MICROARRAY ANALYSIS OF TREATMENT RESPONSE

In the above sections various mechanisms have been described which may be responsible for resistance of tumour cells to cytostatic drugs. Despite the close relationship between expression of the selected proteins and the phenomenon of *in vitro* resistance to cytostatic drugs, the data are not so unequivocal in cases of clinical studies. This may reflect the complexity of the resistance to cytostatic drugs with the effect that determination of individual variables in clinical cases, which are frequently subjected to chemotherapy with the use of several anti-neoplastic agents, often provides no clinically significant information. Another reason for the phenomenon may involve the function of distinct mechanisms, so far unrecognised, responsible for resistance to chemotherapy.

Nowadays, high hopes for more detailed recognition of the phenomenon of resistance to cytostatic drugs and, consequently, for the design of an appropriate predictive clinical test have been placed in DNA microarrays. This technique allows several thousand genes to be examined in a single sample of expression. DNA microarrays thus have the potential for the "blind" examination of several biological phenomena, including MDR. Nevertheless, the technique raises several problems, including that of performing a reliable analysis of such extensive data. The first investigations performed with the use of DNA microarrays have already brought to light new data on the MDR phenomenon [63].

In our study the sensitivity was tested of 30 different cell lines, including four cell lines of ovarian cancer (ES-2, FU-OV-1, OVCAR3 and SKOV-3), to the 11 most frequently applied cytostatic agents in their clinically relevant concentrations [16]. Subsequently, using Affymetrix U133A arrays, the expression profiles of 24,000 genes were tested. For the resistance to each chemotherapy agent an individual prediction profile was constructed, containing 42 to 516 genes. The overall accuracy of the predictions in a leave-one-out cross validation was 81.7%. A list was drawn up identifying the top 67 multidrug resistance candidate genes associated with resistance to at least four anticancer agents. Hartmann et al. [18] described analysis of gene expression using cDNA microarrays containing 30,721 genes in 79 primary surgically resected tumours from women with advanced-stage, high-grade epithelial ovarian cancer. A 14-gene predictive model was developed and subsequently tested. This model correctly predicted the outcome of 24 of the 28 test samples (86% accuracy) with a 95% positive predictive value for early relapse. Spentos et al. [45] in similar studies described a list of 115 genes of independent prognostic significance in ovarian cancer. The authors also provided a list of 93 genes to enable the pathological response in epithelial ovarian cancers to be defined [44]. Jazaeri et al. [23] listed 178 genes, the expression of which differed between ovarian cancer post-chemotherapy samples and primary ovarian tumours. Duan et al. [9] examined three cell lines primarily sensitive to paclitaxel and their variants, which were resistant to the agent. The resistant cell lines differed from the sensitive ones in expression of, respectively, 790, 689 and 964 genes. Summing up, the reports published to date have listed tens to hundreds of new genes which are typical of cases or cell lines resistant to cytostatic drugs.

THE EPIGENETICS OF THE MDR PHENOMENON

In recent years numerous reports have appeared which suggest that disturbed methylation of the so-called CpG DNA islands represents one of the key processes responsible for neoplastic transformation. In promoter regions of structural genes the CpG islands are present in high numbers. Methylation of cytosine induces silencing of a given gene. In tumour cells a significant decrease is noted in global DNA methylation, which results in chromosomal instability. The exception involves promoter regions of suppressor genes which, in contrast, are hypermethylated [10]. Inhibitors of DNA methyltransferase are tested as potential anti-neoplastic drugs [31].

In the period 2003–2006 reports appeared describing the relation between aberrant methylation of individual genes (*MDR1, RASSFIA, HICI, MGMT*) and the development of the resistance of tumour cells to cytostatic drugs. In the light of the fact that methylation aberrations specific to tumours pertain to a significant extent to the genes responsible for the response to cytostatic drugs (genes linked to the response to DNA injury and genes controlling cell cycle apoptosis for instance), the conclusion can be drawn that aberrant methylation also provides grounds for the resistance to cytostatic drugs [31, 38, 53]. The hypothesis is additionally strengthened by the fact that DNA methyltransferase inhibitors make tumour cells sensitive to some drugs (the corresponding literature data pertain mainly to cisplatin) [4].

CONCLUSIONS

The phenomenon of resistance to cytostatic drugs represents a very complex phenomenon and one that remains incompletely recognised. As a result, no predictive panel has yet been designed which could be implemented in routine pathological diagnosis of sensitivity to individual cytostatic drugs in a given patient. The potential for individual prediction of sensitivity to cytostatic drugs is of great importance, since in the first lapse of chemotherapy it enables the application of drugs which are ineffective or poorly effective in tumour cells to be avoided. The application of such drugs results in a growing resistance to chemotherapy in the tumour cells (Fig. 2). When it is taken into account that MDR represents a cross-reactive phenomenon, such cases may be linked to a significantly decreased probability of reaction to drugs, even to agents to which a given tumour was primarily sensitive, following the alteration of therapeutic scheme.

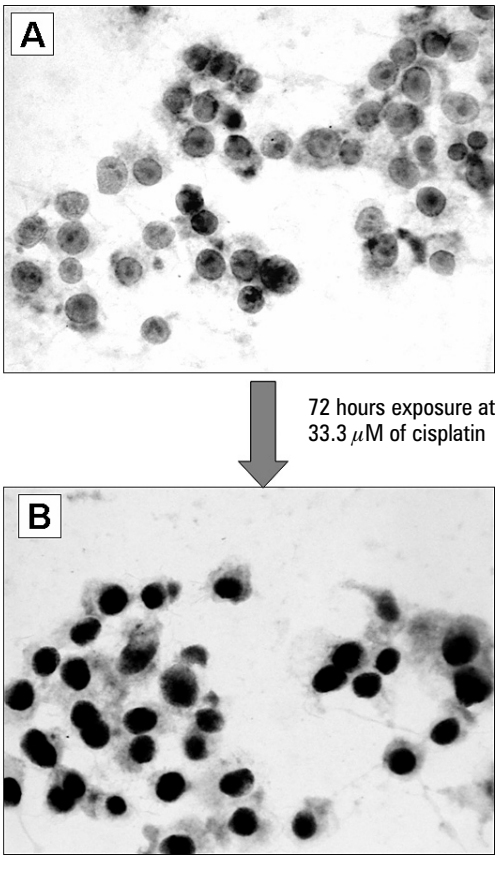

Figure 2. Immunohistochemical localisation of metallothionein expression in: **A.** Cisplatin-resistant A2780RCIS ovarian carcinoma cell line; **B.** Cisplatin-resistant A2780RCIS ovarian carcinoma cell line after 72 hours of exposure at 33.3 μM of cisplatin.

In cases of ovarian cancer tests to determine the expression of p53, BCL-2 and other apoptosis regulators have hitherto had the greatest predictive significance, while the estimation of HER-2, P-gp and the expression of other ABC-transporters are associated with much greater divergence.

Studies using DNA microarrays have brought new insights into the phenomenon of MDR. The reports so far published have listed from tens to hundreds of genes which are typical of cases or cell lines resistant to cytostatic drugs. It is surprising that the lists seldom incorporate genes which have already been recognised as responsible for MDR.

Summing up, the studies performed to date have failed to result in a test which would enable sensitivity or resistance to individual cytostatic agents to be predicted on an individual basis. Such a test cannot rely on the estimation of individual protein expression. It is highly likely that a test to predict the response to chemotherapy in ovarian cancers and other tumours would involve estimation of the expression of more than ten or a hundred

genes and would include the genes which are recognised at present as responsible for MDR as well as new genes selected using DNA microarrays and pangenomic studies on aberrations in DNA methylation.

REFERENCES

1. Allen JD, Schinkel AH (2002) Multidrug resistance and pharmacological protection mediated by the breast cancer resistance protein (BCRP/ABCG2). Mol Cancer Ther, 1: 427–434.
2. Arts HJG, Katsaros D, de Vries EGE, Massobrio M, Genta F, Danese S, Arisio R, Scheper RJ, Kool M, Scheffer GL, Willemese PHB, van der Zee AGJ, Suurmeijer AJH (1999) Drug resistance-associated markers P-glycoprotein, multidrug resistance-associated protein 1, multidrug resistance-associated protein 2, and lung resistance protein as prognostic factors in ovarian carcinoma. Clin Cancer Res, 5: 2798–2805.
3. Averette HE, Hoskins W, Nguyen HN, Boike G, Flessa HC, Chmiel JS, Zuber K, Karnell LH, Winchester DP (1993) National survey of ovarian carcinoma. 1. A patient care evaluation study of the Amercian College of Surgeons. Cancer, 71: 1629–1638.
4. Baker E, El-Osta A (2003) The rise of DNA methylation and the importance of chromatin on multidrug resistance in cancer. Exp Cell Res, 290: 177–194.
5. Beeghly A, Katsaros D, Chen H, Fraccholi S, Zhang Y, Massorbio M, Risch H, Jones B, Yu H (2006) Glutathione S-transferase polymorphisms and ovarian cancer treatment and survival. Gynecol Oncol, 100: 330–337.
6. Brown JM, Attardi LD (2005) The role of apoptosis in cancer development and treatment response. Nat Rev Cancer, 5: 231–237.
7. Cannistra SA (2004) Cancer of the ovary. N Engl J Med, 351: 2519–29.
8. Cullen KJ, Newkirk KA, Schumaker LM, Aldosari N, Rone JD (2003) Glutathione S-transferase pi amplification is associated with cisplatin resistance in head and neck squamous cell carcinoma cell lines and primary tumors. Cancer Res, 63: 8097–8102.
9. Duan Z, Lamendola DE, Cuan Y, Yusuf RZ, Seiden M (2005) Description of paclitaxel resistance-associated genes in ovarian and breast cancer cell lines. Cancer Chemother Pharmacol, 55: 277–285.
10. Esteller M (2005) Aberrant DNA methylation as a cancer-inducing mechanism. Annu Rev Pharmacol Toxicol, 45: 629–656.
11. Fajac A, Da Silva J, Ahomadegbe JC, Rateau JG, Bernaudin JF, Riou G, Benard J (1996) Cisplatin-induced apoptosis and p53 gene status in a cisplatin-resistant human ovarian carcinoma cell line. Int J Cancer, 68: 67–74.
12. Ferrandina G, Fagotti A, Salerno MG (1999) p53 over-expression is associated with cytoreduction and response to chemotherapy in ovarian cancer. Br J Cancer, 81: 733–740.
13. Gottesman MM, Fojo T, Bates SE (2002) Multidrug resistance in cancer: role of ATP-dependent transporters. Nature Rev, 2: 48–58.
14. Gottesman MM (2002) Mechanisms of cancer drug resistance. Annu Rev Med, 53: 615–27.
15. Gumiński AD, Balleine RL, Chiew Y-E, Webster LR, Tapner M, Farrel GC, Harnett PR, DeFazio A (2006) MRP2 (ABCC2) and cisplatin sensitivity in hepatocytes and human ovarian carcinoma. Gynecol Oncol, 100: 239–246.
16. Györffy B, Surowiak P, Kiesslich O, Denkert C, Schäfer R, Dietel M, Lage H (2006) Resistance prediction profile for eleven anticancer agents at clinical concentrations based on the gene expression pattern of thirty cell lines. Int J Cancer, 118: 1699–1712.
17. Hamer DH (1986) Metallothionein. Annu Rev Biochem, 55: 913–951.
18. Hartmann LC, Lu KH, Linette GP, Cliby WA, Kalli KR, Gershenson D, Bast RC, Stec J, Iatchouk N, Smith DI, Ross JS, Hoersch S, Shridhar V, Lillie J, Kaufmann SH, Clark EA, Damokosh AI (2005) Gene expression profiles predict early relapse in ovarian cancer after platinum-paclitaxel chemotherapy. Clin Cancer Res, 11: 2149–2155.
19. Havrilesky L, Darcy KM, Hamdan H (2003) Prognostic significance of p53 mutation and p53 overexpression in advanced epithelial ovarian cancer: A Gynecologic Oncology Group Study. J Clin Oncol, 21: 3814–3825.
20. Hishikawa Y, Abe S, Kinugasa S, Yoshimura H, Monden N, Igarashi M, Tachibana M, Nagasue N (1997) Overexpression of metallothionein correlates with chemoresistance to cisplatin and prognosis in esophageal cancer. Lab Invest, 54: 342–347.
21. Husain I, Mohler JL, Seigler HF, Besterman JM (1994) Elevation of topoisomerase I messenger RNA, protein, and catalytic activity in human tumors: demonstration of tumor-type specificity and implications for cancer chemotherapy. Cancer Res, 54: 539–546.
22. Ikeda K, Sakai K, Yamamoto R, Hareyama H, Tsumura N, Watari H, Shimizus M, Minanami H, Sakuragi N (2003) Multivariate analysis for prognostic significance of histologic subtype, GST-pi, MDR-1, and p53 in stages II–IV ovarian cancer. Int J Gynecol Cancer, 13: 776–784.
23. Jazaeri AA, Awtrey CS, Chandramouli GVR, Chuang YE, Khan J, Sotiriou C, Aprelkova O, Yee CJ, Zorn KK, Birrer MJ, Barrett JC, Boyd J (2005) Gene expression profiles associated with response to chemotherapy in epithelial ovarian cancer. Clin Cancer Res, 11: 6300–6310.
24. Jia P, Wu S, Li F, Xu Q, Wu M, Chen G, Liao G, Wang S, Zhou J, Lu Y, Ma D (2005) Breast cancer resistance protein-mediated topotecan resistance in ovarian cancer cells. Int J Gynecol Cancer, 15: 1042–1048.
25. Katsaros D, Fracchioli S, Arts HJ, de Vries EG, Danese S, Richiardi G, Arisio R, Gordini G, van der Zee AG, Suurmeijer AJ, Massobrio M (1999) Expression and prognostic value of the drug resistance markers P-gp, Mrp1, Mrp2, and Lrp in ovarian carcinoma. Minerva Ginecol, 51: 463–470.
26. Kawiak J, Zabel M (2002) Seminaria z cytofizjologii. Urban & Partner, Wrocław.
27. Kool M, de Haas M, Scheffer GL, Scheper RJ, van Eijk MJ, Juijn JA, Baas F, Borst P (1997) Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. Cancer Res, 57: 3537–3547.

28. Kowalski P, Surowiak P, Lage H (2005) Reversal of drug--resistant phenotypes by an autocatalytic multitarget multirybozyme directed against the transcripts of the ABC transporters MDR1/P-gp, MRP2, and BCRP. Mol Ther, 11: 1393–1398.
29. Kupryjanczyk J, Bell D, Dimeo D (1995) p53 gene analysis of ovarian bordeline tumors and stage I carcinomas. Hum Pathol, 26: 387–392.
30. Liedert B, Materna V, Schadendorf D, Thomale J, Lage H (2003) Overexpression of cMOAT (MRP2/ABCC2) is associated with decreased formation of platinum-DNA adducts and decreased G2-arrest in melanoma cells resistant to cisplatin. J Invest Dermatol, 121: 172–176.
31. Lyko F, Brown R (2005) DNA methyltransferase inhibitors and the development of epigenetic cancer therapies. J Natl Cancer Inst, 97: 1498–1506.
32. Materna V, Pleger J, Hoffmann U, Lage H (2004) RNA expression of MDR1/P-glycoprotein, DNA-topoisomerase I, and MRP2 in ovarian carcinoma patients: correlation with chemotherapeutic response. Gynecol Oncol, 94: 152–160.
33. McCluggage WG, Strand K, Abdulkair A (2002) Immunohistochemical localization of metallothionein in benign and malignant epithelial ovarian tumours. Int J Gynecol Cancer, 12: 62–65.
34. McLeod HL, Douglas F, Oates M, Symonds RP, Prakash D, van der Zee AG, Kaye SB, Brown R, Keith WN (1994) Topoisomerase I and II activity in human breast, cervix, lung and colon cancer. Int J Cancer, 59: 607–611.
35. Patel VA, Dunn MJ, Sorokin A (2002) Regulation of MDR1 (P-glycoprotein) by cyclooxygenase-2. J Biol Chem, 227: 38915–38920.
36. Perego P, Gatti L, Righetti SC, Bereta GL, Carenini N, Corna E, Bo LD, Tineli S, Cloangelo D, Leone R, Apostoli P, Lombardi L, Beggiolini G, Piazzoni L, Zunino F (2003) Development of resistance to a trinuclear platinum complex in ovarian carcinoma cells. Int J Cancer, 105: 617–624.
37. Plisiecka-Hałasa J, Karpińska G, Szymańska T, Ziółkowska I, Mądry R, Timorek A, Dębniak J, Ułańska M, Jedryka M, Chudecka-Głaz A, Klimek M, Rembiszewska A, Kraszewska E, Dybowski B, Markowska J, Emerich J, Płużańska A, Goluda M, Rzepka-Górska I, Urbański K, Zieliński J, Stelmachów J, Chrabowska M, Kupryjańczyk J (2003) $P21^{WAF1}$, $p27^{KIP1}$, TP53 and C-MYC analysis in 204 ovarian carcinomas treated with platinum-based regimens. Annals Oncol, 14: 1078–1085.
38. Qiu YY, Mirkin BL, Dwivedi RS (2005) Inhibition of DNA methyltransferase reverses cisplatin induced drug resistance in murine neuroblastoma cells. Cancer Det Prev, 29: 456–463.
39. Rodriguez-Galindo C, Radomski K, Stewart CF, Furman W, Santana VM, Houghton PJ (2000) Clinical use of topoisomerase inhibitors in anticancer treatment. Med Ped Oncol, 35: 382–402.
40. Sarkadi B, Ozvegy-Laczka C, Nemet K, Varadi A (2004) ABCG2 — a transporter for all seasons. FEBS Letters, 567: 116–120.
41. Satoh T, Nishida M, Tsunoda H, Kubo T (2001) Expression of glutathione S-transferase pi (GST-pi) in human ovarian tumors. Eur J Obstet Gynecol Reprod Biol, 96: 202–208.
42. Siddik ZH (2003) Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene, 22: 7265––7279.
43. Slamon D, Clark G (1988) Amplification of C-ERB-B2 and agressive breast tumors? Science, 240: 1795–1798.
44. Spentoz D, Levine DA, Kolia S, Out H, Boyd J, Libermann TA, Cannistra SA (2005) Unique gene expression profile based on pathologic response in epithelial ovarian cancer. J Clin Oncol, 23: 7911–7917.
45. Spentoz D, Levine DA, Ramoni MF, Joseph, M, Gu X, Boyd J, Lbermann TA, Cannistra SA (2004) Gene expression signature with independent prognostic significance in epithelial ovarian cancer. J Clin Oncol, 22: 4700–4710.
46. Surowiak P, Kaplenko I, Spaczyński M, Zabel M (2003) Expression of metallothionein (MT) and intensity of proliferation in ovarian cancers treated with cisplatin and paclitaxel. Fol Morphol, 62: 493–495.
47. Surowiak P, Materna V, Denkert C, Kaplenko I, Spaczynski M, Dietel M, Zabel M, Lage H (2006) Significance of cyclooxygenase 2 and MDR1/P-glycoprotein coexpression in ovarian cancers. Cancer Letters, 235:272–280.
48. Surowiak P, Materna V, Kaplenko I, Spaczynski M, Dietel M, Lage H, Zabel M (2005) Augmented expression of metallothionein and glutathione S-transferase pi as unfavorable prognostic factors in cisplatin-treated ovarian cancer patients. Virchows Archiv, 447: 626––633.
49. Surowiak P, Materna V, Kaplenko I, Spaczynski M, Dietel M, Lage H, Zabel M (2006) Topoisomerase 1A, HER/2neu and Ki67 expression in paired primary and relapse ovarian cancer tissue samples. Histol Histopathol, 25: 131–139.
50. Surowiak P, Materna V, Kaplenko I, Spaczyński M, Dolińska-Krajewska B, Gębarowska E, Dietel M, Zabel M, Lage H (2006) ABCC2 (MRP2, cMOAT) can be localized in the nuclear membrane of ovarian carcinomas and correlates with resistance to cisplatin and clinical outcome. Clin Cancer Res [in press].
51. Surowiak P, Materna V, Matkowski R, Szczuraszek K, Kornafel J, Wojnar A, Pudelko M, Dietel M, Denkert C, Zabel M, Lage H (2005) Relationship between cyclooxygenase 2 and P-glycoprotein expressions in invasive breast cancers and their prognostic significance. Breast Cancer Res, 7: R862–R870.
52. Taniguchi K, Wada M, Kohno K, Nakamura T, Kawabe T, Kawakami M, Kagotani K, Okumura K, Akiyama S, Kuwano M (1996) A human canalicular multispecific organic anion transporter (cMOAT) gene is overexpressed in cisplatin-resistant human cancer cell lines with decreased drug accumulation. Cancer Res, 56: 4124–4129.
53. Teodoridis JM, Strathdee G, Plumb JA, Brown R (2004) CpG-island methylation and epigenetic control of resistance to chemotherapy. Biochem Soc Transactions, 32: 916–917.
54. Verkis A, Meynard D, Haaz M-C, Bayssas M, Bonnet J, Robert J (2004) Molecular determinants of the cytotoxicity of platinum compounds: the contribution of in silico research. Cancer Res, 64: 356–362.

55. Wang D, Lippard SJ (2005) Cellular processing of platinum anticancer drugs. Nature Reviews. Drug Discovery, 4: 307–320.
56. Wang JC (2002) Cellular roles of DNA topoisomerases: a molecular perspective. Nat Rev Mol Cell Biol, 3: 430–440.
57. Wernyj RP, Morin PJ (2004) Molecular mechanisms of platinum resistance: still searching for the Achilles' heel. Drug Resistance Updates, 7: 227–232.
58. Williams J, Lucas PC, Griffith KA, Choi M, Fogoros S, Hu YY, Liu R (2005) Expression of Bcl-x_L in ovarian carcinoma is associated with chemoresistance and recurrent disease. Gynecol Oncol, 96: 287–295.
59. Wirgley E, Verspaget HW, Jayson GC, McGown AT (2000) Metallothionein expression in epithelial ovarian cancer: effect of chemotherapy and prognostic significance. J Cancer Res Clin Oncol, 126: 717–721.
60. Yakirevich E, Sabo E, Naroditsky I, Sova Y, Lavie O, Resnick MB (2006) Multidrug resistance-related phenotype and apoptosis-related protein expression in ovarian serous carcinomas. Gynecol Oncol, 100: 152–159.
61. Yoh K, Ishii G, Yokose, T, Minegishi Y, Tsuta K, Goto K, Nishiwaki Y, Kodama T, Suga M, Ochiai A (2004) Breast cancer resistance protein impacts clinical outcome in platinum-based chemotherapy for advanced non-small cell lung cancer. Clin Cancer Res, 10: 1691–1697.
62. Zabel M, Kaczmarek A, Ramlau R, Matecka-Nowak M (1991) Rola P-glikoproteiny w wielolekowej oporności nowotworów na cytostatyki. Nowotwory, 41: 32–45.
63. Zasławski R, Surowiak P, Zabel M (2005) Classical and novel molecular prognostic factors in ovarian cancers. Pol Merk Lek, 114: 105–111.