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Review

Prediction of Drug Sensitivity and Resistance of Cancer by Protein Expression Profiling

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Abstract. *Although the statistical probability of therapeutic success is known for larger groups of cancer patients, the clinical response to chemotherapy of the individual patient remains uncertain. It would be of great value to know whether or not an individual tumor responds to the proposed therapy. The concept of sensitivity testing of tumors for individualized therapy traces back to the 1970s. Currently, an astonishing revival has taken place due to the thriving development of genomic and proteomic technologies. This review discusses our own results on protein expression profiles of non-small cell lung cancer, kidney carcinoma and acute lymphoblastic leukemia regarding the prediction of drug sensitivity or resistance. A great diversity of drug resistance mechanisms are operative in the clinical drug resistance of cancer e.g., resistance proteins, proliferative, apoptotic, angiogenic factors, proto-oncogenes and tumor suppressor-genes. Hierarchical cluster analyses and cluster image maps reveal different resistance profiles even within cancer types of homogeneous histology. Protein arrays may be appropriate to perform sensitivity or resistance tests for individual patients because thousands of proteins may be detected in a single experiment. On the other hand, results suggest that already a set of a limited number of factors may be sufficient to detect the sensitivity or resistance of a cancer.*

Abbreviations: P-gp (MDR1, ABCB1), P-glycoprotein; GST-pi, glutathione S-transferase-pi; MGMT, O^6-methylguanine-DNA-methyl-transferase; MT, metallothionein; MVP/LRP, major vault protein (lung resistance-related protein); TS, thymidylate synthetase; PCNA, proliferating cell nuclear antigen; VEGF, vascular endothelial growth factor; PD-ECGF, platelet-derived endothelial cell growth factor; FLT1, fms-related tyrosine kinase I (vascular endothelial growth factor / vascular permeability factor receptor); FAS (CD95), tumor necrosis factor receptor; NM23, protein expressed in non-metastatic cells; AP, alkaline phosphatase; PKC, protein kinase C.

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Historical Remarks about Predictive Tests

Resistance to chemotherapy is a major source of failure in cancer treatment. Since the advent of the first cytostatic drug (1), this problem has dogged oncology for more than half a century without satisfying solutions. A large number of cancers are intrinsically resistant to cytostatic agents. Other tumors, initially responding to treatment, develop drug resistance during chemotherapy. Therefore, the question is which particular cytostostatic agent or which combination of substances are most suited for an individual tumor. While the statistical probability of therapeutic success is well-known for larger groups of patients from clinical therapy trials, it is, however, not possible to predict which individual tumor will respond to chemotherapy. It would be, therefore, of great value for patients to know, whether or not a tumor will respond to the proposed therapy. If the tumor is resistant, the therapy will cause only toxic effects in normal tissues without influence on the tumor growth. Therefore, the concept of sensitivity testing of tumors for individualized therapy was launched in the 1970s (2-5). The idea was to determine *in vitro* the response of tumors to cytostatic drugs beforehand, in order to choose the most effective treatment for each patient clinically. The methods available at that time, however, did not find widespread application in clinical routine diagnostics. In the 1990s, attempts were made to test *a priori* drug response of tumors by assessing the expression of resistance proteins (6). However, it was difficult to define consensus recommendations for the standardized detection of resistance proteins expressed in low amounts in tumors with low degrees of drug resistance (7-9). Another important

reason was that no single mechanism can explain resistance to therapy (10). The sequencing of the human genome opened new avenues, not only for our understanding of the complex network of genes in cancer cells but also for new technological developments (11-13). Micro-arrays allow the simultaneous analysis of thousands of genes or proteins in a single experiment. Hence, it comes as no surprise that the old concept of prediction of drug response and individualized therapy is currently experiencing a thriving revival.

The Multifactorial Nature of Cancer Drug Resistance

Resistance proteins. One class of drug resistance phenotypes that has now been well characterized is the so-called multidrug resistance (MDR) (14). Investigations conducted with tumor cell lines and tumor specimens obtained from cancer patients have shown that cross-resistance between different drugs that are structurally and functionally dissimilar is a common phenomenon. The MDR phenomenon is associated with the overexpression of members of the ATP-binding cassette (ABC) transporter gene family (15). The first ABC transporter to be identified in drug-resistant cells was a 170 kDa membrane-associated glycoprotein (P-gp) that decreases intracellular drug accumulation (16). The expression of this protein results in resistance to a variety of anticancer drugs such as vinca alkaloids, anthracyclines, anthracendiones, epipodophyllotoxins and taxanes. But the overexpression of P-gp alone does not completely explain all variants of multidrug-resistant phenotypes. During recent years, other ABC-transporters have been identified that also confer MDR (17). Another group of proteins - controversially discussed in the context of MDR - are the major vault proteins (MVP). The human homologue of MVP was initially termed lung resistance protein (LRP) (18). Other proteins contribute to the pleiotropic drug resistance phenomena as well (19,20). Thus, the term MDR should be extended to all phenomena of cross-resistance of tumor cells to drugs of chemically and / or functionally unrelated classes.

Topoisomerase II is a ubiquitous nuclear enzyme that is essential for replication and transcription. This enzyme is the target of many anti-neoplastic drugs such as anthracyclines, amsacrines and epipodophyllotoxin (21). Tumor cells acquire drug resistance either by protein down-regulation or by point mutation-related enzymatic alterations. O^6-methylguanine-DNA-methyltransferase (MGMT) is a ubiquitous DNA repair protein and MGMT-deficient cells decrease the sensitivity to alkylating agents (22, 23). Thymidylate synthetase (TS) is the target of many chemotherapeutic agents, such as 5-fluorouracil and methotrexate. Tumor cells that are resistant to antimetabolites, cisplatin or doxorubicin have increased levels of this enzyme (24, 25). Glutathione and glutathione-S-transferases (GST) play an important role in the detoxification of cytostatic compounds. Melphalan, cyclophosphamide, chlorambucil and doxorubicin are substrates for these proteins (26). Another important protein for drug resistance is metallothionein (MT), that binds heavy metal ions such as zinc, copper, cadmium and platinum. It inactivates metal-containing anticancer agents (27). Dihydrofolate reductase (DHFR) is the primary target for the action of antifolate drugs in cancer chemotherapy. It has been identified as an important mechanism for methotrexate resistance (28). Heat shock proteins (HSPs) are a family of proteins that protect cells from toxic external stimuli (29). Cells that overexpress heat shock proteins are resistant to doxorubicin, colchicine and vincristine.

The influence of proto-oncogenes and suppressor genes. There is compelling evidence that proto-oncogenes are involved in drug resistance (20, 30, 31). These results unify the mechanisms responsible for carcinogenesis and for drug resistance on a common molecular basis (20, 32-34). Tumors induced by activation of proto-oncogenes or inactivation of tumor suppressor genes may, thus, also be resistant to therapy providing a molecular model for intrinsic drug resistance of tumors. In several types of tumors, it has been confirmed that bcl-2-negative tumors are more often sensitive to anticancer drugs than bcl-2-positive tumors (35). P53 also plays an important role in resistant tumors (36). Growth factor receptors (ErbB1, ErbB2), signal transducers (Ras) and transcription factors (Fos, Jun, Myc) affect cellular response to cytostatic drugs by regulation of apoptosis, DNA repair or the induction of resistance gene expression (31, 37, 38). While all of the above-mentioned proteins contribute to the complex phenomenon of drug resistance none of them alone is able to explain it (10).

The influence of proliferative, apoptotic and angiogenic factors. It is generally accepted that cancer chemotherapy is most successful when used on rapidly growing malignant cells (39). Experimental and clinical data show that tumors with a low rate of proliferation are less responsive to treatment than tumors with a high rate of proliferation (40,41). Apoptosis represents another important determinant for the response of tumors to cytostatic agents (42). This process involves the death-inducing ligand receptor systems and the cleavage of caspases (43, 44). Angiogenesis, the development and formation of new blood vessels, plays an important role in a variety of processes including resistance. Solid tumors with few blood vessels contain hypoxic cells that are relatively resistant to radiotherapy and certain cytostatic drugs (45, 46). Glutathione S-transferase-pi, thymidylate synthetase, metallothionein and, with some restriction, P-glycoprotein were overexpressed in tumors with poor vascularization (47). The vascular endothelial growth factor (VEGF) and the fibroblast growth factor (FGF) are molecules that directly exert an angiogenic effect

Table I. *In vitro-in vivo correlation of various predictive test systems.*

	(n)	Correctly diagnosed (%)	Sensitivity (%)	Resistance (%)
Tissue culture	676	62	45	96
Short-term test	664	88	78	94
Agar cloning assay	302	90	61	97

According to Volm (61).

and might also influence the therapeutic response of tumors (48, 49). Expression of platelet-derived endothelial cell growth factor (PD-ECGF) is elevated in several types of tumors and plays a role in tumor vascularisation and drug response (50). Tissue factor (TF), a physiological initiator of blood coagulation, is also involved in tumor growth and angiogenesis and influences drug resistance (51).

As more and more factors that contribute to drug resistance were identified during recent years, the entire complexity of multifactorial drug resistance has become evident. For the prediction of drug resistance in clinical routine diagnostics, it is obviously not sufficient to investigate single genes or proteins. At this point, holistic analyses of an entire battery of genes or proteins conferring drug resistance may be more promising to gain deeper insight into the full potential of unresponsive tumors. All these above-mentioned factors regarding drug resistance have been investigated by our group during the past decade in order to prove which factors are important for clinical drug resistance.

Clinical relevance of different predictive tests. Comparable to the culture techniques in microbial chemotherapy, it has been attempted to determine the resistance of tumor cells in experimental models. The literature refers to sensitivity tests, resistance tests or oncobiograms (52, 53). The following approaches have been investigated in the past:

a) measurement of cellular damage in tissue or organ culture (54, 55);

b) measurement of the inhibition to radioactive precursor incorporation (short-term test) (3, 4, 56);

c) measurement of clonogenic cell survival (2, 57); and

d) measurement of human tumor xenografts (58, 59).

The overall practical value of a test system depends on how reliably the results of clinical therapy can be predicted. This capacity (*in vitro-in vivo* correlation) was established in two-thirds of all cases in the tissue culture method. A better correlation was provided by the clonogenic assay and the short-term test. All these systems had in common a better ability to detect drug resistance (94-97%) than drug sensitivity (45-78%) (60, 61) (see Table I). A completely accurate prediction of the response of a tumor could not be achieved by any available test system, since the complexity cannot be defined sufficiently by the measurement of a single parameter. Therefore, the micro-array technologies may be more suitable for predictive tests of individuals. Profiling gene expression using mRNA micro-arrays has had an important impact on biomedical research and has proven to be a very powerful tool for the multiplexed comparative analysis of gene expression (62). Gene array analysis is a rapid way to compare the expression levels of thousands of mRNA species simultaneously in normal *vs.* tumor tissues or treated *vs.* untreated cells. Up to now, most clinical studies with mRNA micro-arrays have examined pathologically homogeneous sets of tumors to identify clinically relevant subtypes. It was found that gene expression profiling was a more powerful predictor of disease outcome in patients with cancer than clinical and histological factors (63-66). Scherf *et al.* (11) carried out the first study to integrate large databases of gene expression and molecular pharmacology. They used cDNA micro-arrays to explore the expression of approximately 8,000 unique genes among the 60 cell lines used in the National Cancer Institute's (NCI) screen for anticancer drugs. They correlated gene expression and drug activity pattern in the 60 cell lines. Gene-drug relationships for the agents 5-fluorouracil and L-asparaginase exemplify how variations in the transcript levels of particular genes relate to mechanisms of drug sensitivity. Staunton and coworkers (67) determined whether the gene expression signatures of untreated cells are sufficient for the prediction of drug sensitivity. Using the panel of the 60 human cancer cells lines, gene expression-based classifiers of sensitivity or resistance for 232 compounds were generated. The accuracy of drug sensitivity prediction was considerably better than would be expected by chance. Eighty-eight of 232 expression-based classifiers performed accurately on an independent test set. This suggests that, at least for a subset of compounds, genomic appproaches to chemosensitivity prediction are feasible. To explore genes that determine the sensitivity of cancer cells to cytostatic agents, Dan *et al.* (68) investigated the expression of about 9,000 genes using cDNA microarrays in 39 human cancer cell lines. Whereas some genes commonly correlated with various classes of anticancer drugs, other genes correlated only with specific drugs with similar mechanisms. Zembutsu and coworkers (69) used a cDNA micro-array representing over 23,000 genes to analyze expression profiles in a panel of 85 cancer xenografts in nude mice. These xenografts were derived from 9 human organs. The xenografts were examined for sensitivity to 9 anticancer drugs. Comparison of the gene expression profiles of the tumors with sensitivities to each drug identified 1,570 genes whose expression levels correlated significantly with drug sensitivity. Three hundred

and thirty-three of those genes showed a significant correlation with two or more drugs and 32 genes correlated with 6 or 7 drugs.

The possibility of performing similar analyses at the protein level is, therefore, very attractive, and profiling studies of disease tissue with protein assays are beginning to emerge rapidly (70-76). However, proteome analysis is at a much earlier stage of development than gene expression studies. Comparable to the robot-aided spotting of cDNAs or oligonucleotides onto glass slides to construct mRNA micro-arrays, antibodies, ligands, or peptides can be put onto solid phases either in micro-array or in ELISA-based formats. The combination of tissue array technology with standard methods such as immunohistochemistry or interphase *in situ* hybridization allows high-throughput analyses of both archived and fresh tumor biopsies. The combination of multidimensional chromatography, mass spectrometry and differential protein expression approaches enables the automated short-term investigation of thousands of differentially expressed proteins. Protein-protein interaction, signal transduction pathways and other network analyses are expected to be unravelled in a comparable manner.

Monoclonal antibodies are currently the preferred choice for protein capture agents due to their high specificity, affinity and stability. The ideal protein micro-array would consist of a large number of high affinity, high specificity protein ligands, one for each protein in the proteome of interest. But this means that 100,000 to 1,000,000 validated monoclonal antibodies have to be at hand. Therefore, a reasonable intermediate goal would be to construct arrays of 50 to 100 protein-binding ligands directed against proteins which are important for predictive tests (for example resistance-related proteins, proto-oncogenes, tumor suppressor genes, proliferative, apoptotic and angiogenic factors).

Two-dimensional gel electrophoresis was already being used in cellular and molecular biology long before the term proteomics was ever coined. It has developed recently to a sophisticated methodology equipped with software programs for the evaluation and documentation of protein spots and supplemented with peptide fingerprinting, mass spectrometry and worldwide accessible amino acid sequence databases for the identification of proteins. A systematic approach to study drug resistance by proteomics was undertaken by Sinha and coworkers (77-79). The following groups of proteins have been involved in resistant cell lines: proteins associated with signal transduction, HSP and other chaperones, enzymes involved in metabolic pathways, calcium-binding proteins, proliferation markers, modulators of protein kinase C activity, proteins affecting gene expression, cytoskeletal proteins and proteins involved in drug detoxification. Protein arrays may be more appropriate for predictive tests than DNA- or mRNA-arrays, because DNA- and mRNA-based analyses provide only an indirect measure and these do not always reflect protein levels accurately. Moreover, the activities of many proteins are affected by post-translational modifications such as phosphorylation, glycosylation and acetylation.

Table II. *Sensitivity and specificity analysis of the most significant resistance factors.*

factor1 factor2 factor 3	Sp (%)	Se (%)	Se+Sp (%)	CD (%)
Single parameter analysis:				
TS	81.69	60.87	142.56	76.60
VEGF	79.37	57.89	137.26	74.39
GST-pi	73.24	69.57	142.80	72.34
MT	71.43	60.87	132.30	68.82
P-gp	59.15	91.30	150.46	67.02
FGF	42.62	90.00	132.62	54.32
Double parameter analysis :				
VEGF GST-pi	92.06	63.16	155.22	85.37
VEGF P-gp	84.13	84.21	168.34	84.15
FGF P-gp	78.69	85.00	163.69	80.25
VEGF FGF	63.79	83.33	147.13	68.42
P-gp TS	54.93	100.00	154.93	65.96
P-gp GST-pi	56.34	95.65	151.99	65.96
Triple parameter analysis:				
VEGF FGF P-gp	91.38	83.33	174.71	89.47
VEGF GST-pi MT	82.26	78.95	161.21	81.48
VEGF P-pg TS	77.78	89.47	167.25	80.49
VEGF P-gp MT	70.97	94.74	165.70	76.54
FGF P-gp TS	70.49	95.00	165.49	76.54
FGF P-pg GST-pi	70.49	90.00	160.49	75.31

Se= Sensitivity; Sp= Specificity (Resistance); CD= Correctly diagnosed (according to Volm and Rittgen (80)).
P-gp=P-glycoprotein; GST-pi=glutathione S-transferase-pi; TS=thymidylate synthetase; VEGF=vascular endothelial growth factor; FGF= fibroblast growth factor; MT=metallothionein.

Own Results

Lung cancer. Contribution of individual factors to drug resistance. The main objective of our studies was to evaluate which cellular factors are most predictive for the resistance exhibited by non-small cell lung carcinomas and whether or not a combination of factors can improve the prediction of drug sensitivity or drug resistance. Ninety-four patients with previously untreated non-small cell lung carcinomas were admitted into this study. Significant correlations were detected between the data obtained by the *in vitro* short-term test for determining the drug resistance and expressions of proteins by immunohistochemistry. In our previous analyses (80) a significant relationship of resistance was obtained with P-glycoprotein (P-gp, $p=0.00004$), glutathione-S-transferase-

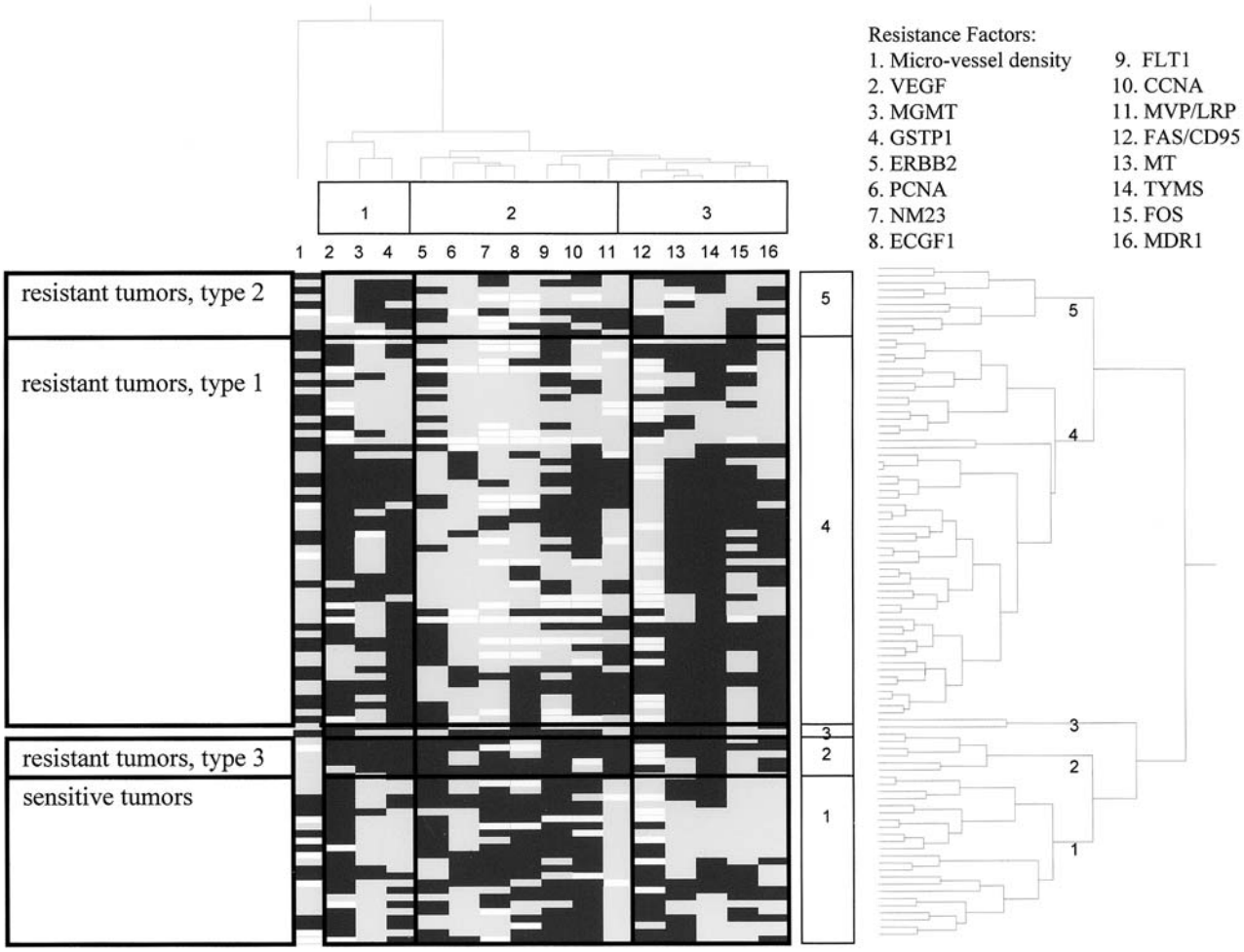

Figure 1. *Dendrogram of hierarchical cluster analysis (complete linkage method) and clustered image map (CIM) obtained from protein expressions in primary non small cell lung carcinomas of 94 patients.*
Light fields, low or absent protein expression, dark fields, strong protein expression, white fields, missing values.
(according to (81)).

pi (GST-pi, $p=0.0002$), metallothionein (MT, $p=0.0008$), thymidylate synthase (TS, $p=0.002$), O^6-methylguanine-DNA-methyltransferase (MGMT, $p=0.008$) and lung resistance protein (LRP, $p=0.03$). A weak correlation existed with the heat shock proteins HSP70 ($p=0.05$). A weak relationship also existed between the expressions of cdk2 ($p=0.04$) and PCNA ($p=0.05$) and the *in vitro* resistance of lung cancer to doxorubicin. We found a significant relationship of resistance to the Fas receptor (CD95, $p=0.007$). ErbB-2 correlated with drug resistance ($p=0.04$). Platelet-derived endothelial growth factor (PD-ECGF, $p=0.0006$), vascular endothelial growth factor (VEGF, $p=0.004$) and fibroblast growth factor (FGF, $p=0.007$) exhibited significant inverse correlations to the resistance of non-small cell lung cancer. Summing up, these analyses showed that the drug resistance proteins are the most important factors associated with the resistance of non-small cell lung cancer. Angiogenic and apoptotic factors are of secondary importance. In contrast, the predictive value of the proliferative factors and proto-oncogenes is only marginal at best. An inverse relationship exists between angiogenic or apoptotic factors and drug resistance proteins.

Using a statistical sensitivity/specificity test (80) the diagnostic accuracy of all parameters was calculated. In Table II only the six best factors have been presented. About 77% of the tumors could be correctly diagnosed with thymidylate synthetase (TS). To determine whether a combination of factors could yield improved information for prediction of drug resistance and sensitivity, the sensitivity and specificity of all pairs of factors were evaluated. The best prediction is attained with a combination of VEGF and GST-pi; 85.4 % of the tumors could be diagnosed correctly.

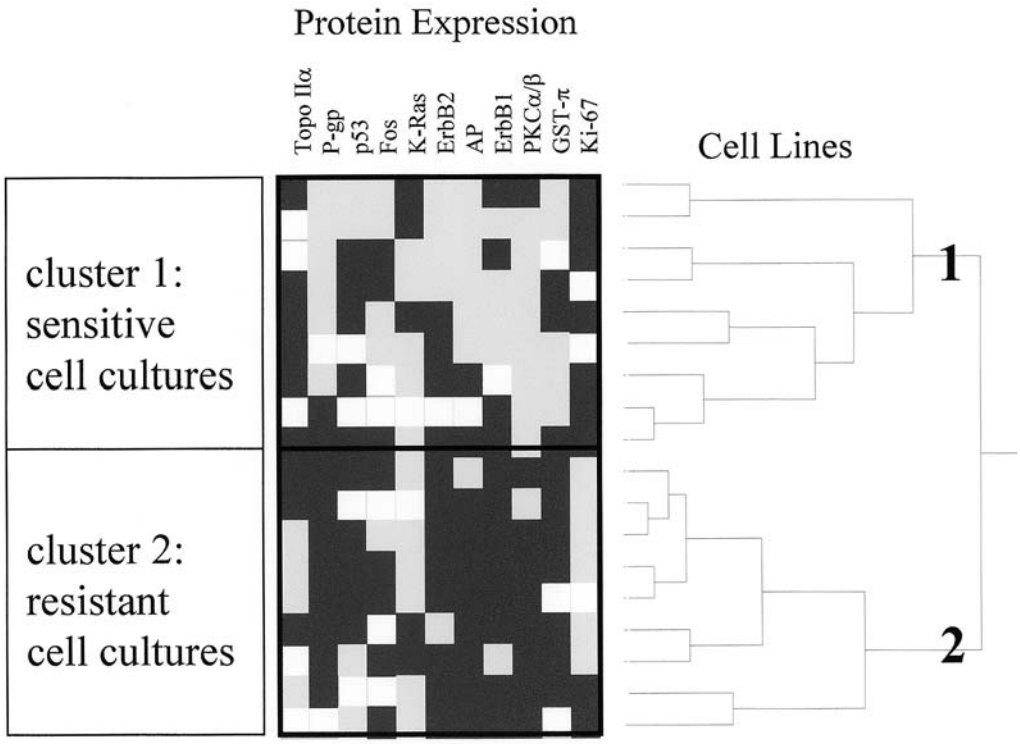

Figure 2. *Dendrogram of hierarchical cluster analysis and clustered image map obtained from protein expressions in kidney cell cultures of 18 patients. Light fields, low or absent protein expression; dark fields, strong protein expression; white fields, missing values. (according to (82)).*

Similarly, triplets were tested. The best results were achieved by the combination of VEGF, FGF and P-gp with 89.47% of the tumors diagnosed correctly (Table II). Thus, the systematic investigation of combinations of cellular factors in lung cancer yields an improvement in the predictive information. Using three factors the responsiveness to drugs exhibited by the carcinomas could be correctly diagnosed in up to nearly 90% of the cases.

Hierarchical cluster analyses. The analysis described above, does not allow a deeper insight into the higher complexity of the multifactorial nature of drug resistance. Therefore, we performed hierarchical cluster analyses, which may be more suited to unravel the full potential of such data sets. Hierarchical cluster analysis is an explorative statistical method and aims to group at first sight heterogeneous objects into clusters of homogeneous objects. All objects are assembled into a cluster tree (dendrogram, Figure 1, Figure 2). Objects with tightly related features appear together, while the separation in the cluster tree increases with progressive dissimilarity. To construct clustered-image maps (CIM), two dendrograms were related to each other (Figure 1, Figure 2). The resistance factors were cluster-ordered on the basis of their expression pattern across the lung carcinomas of 94 patients (Figure 1) and the kidney cell cultures of 18 patients (Figure 2). Thus resistance parameters with the most nearly identical pattern appear side by side on the x-ordinate. *Vice versa*, the carcinomas were cluster-ordered according to the protein expression. Tumors with the most identical expression pattern of resistance factors appear side by side on the y-co-ordinate.

We subjected only those 16 proteins of the lung carcinomas to hierarchical cluster analyses which showed a significant relationship to resistance, in order to find out expression profiles indicative for drug resistance of lung cancer. The dendrogram obtained by this analysis could be divided into four different clusters (Figure 1). One cluster was enriched with sensitive carcinomas while the other clusters obtained resistant carcinomas. These clusters showed only a relationship to drug response but not to other clinical parameters. Among the three clusters that showed an enrichment of resistant tumors, three different expression profiles became apparent (Figure 1). In the most prevalent expression profile, all drug resistance proteins investigated were up-regulated (P-gp, TS, GST-pi, MT, MGMT, LRP). Microvessel density and the angiogenic

factors VEGF, FLT1 and PD-ECGF were down-regulated. Additionally, the proliferative factors PCNA and cyclin A were reduced. The apoptotic factor FAS/CD95 was less expressed than in sensitive carcinomas. Of the proto-oncogenes and suppressor genes FOS was up-regulated, while NM23 and ErbB2 were down-regulated. In the second expression profile, only three of six investigated resistance proteins were up-regulated (GST-pi, MGMT, LRP). Again microvessel density was reduced and the angiogenic factors (VEGF, PD-ECGF) were more down-regulated than those of the first expression profile with resistant tumors. The proliferative and apoptotic factors were reduced. In the third resistance profile only five resistance proteins were increased, while the other resistance-associated parameters revealed only marginal changes (81).

Kidney carcinoma. Kidney carcinomas are mainly treated by surgery and radiotherapy due to their frequent drug resistance. Therefore, kidney tumors represent a suitable model to study drug resistance phenomena. While we analyzed primary human lung cancer, we now analyze human primary cell cultures of renal cell carcinomas of 18 patients. First, we determined the sensitivity to doxorubicin, vincristine and mafosfamide (an *in vitro* active derivate of cyclophosphamide). Then, the cell cultures were analyzed by immunohistochemistry and the expression levels of the proteins were correlated to drug response. Only those proteins that showed a relationship to resistance (P-gp, GST-pi, Topo II, PKC, AP, ErbB1, ErbB2, Fos, K-Ras, p53, Ki67) were analyzed by hierarchical cluster analysis (Figure 2). The obtained dendrogram could be separated in two main clusters which defined cell cultures as sensitive or resistant both to doxorubicin and vincristine. The resistant cell cultures showed an up-regulation of P-gp, GST-pi, PKC, AP and a down-regulation of Topo II in comparison to sensitive cell cultures. The expression of ErbB1, ErbB2 and Fos was higher in resistant cell cultures compared to sensitive cell cultures, while K-Ras expression was reduced in resistant cell cultures. Finally, a clustered image map was generated (Figure 2). The sensitive area was characterized by a lower expression of drug- resistant factors than the resistant area (82).

Childhood acute lymphoblastic leukemia (ALL). In contrast to lung and kidney cancer, acute lymphoblastic leukemia cells (ALL) of children respond frequently to chemotherapy. Children with ALL treated by chemotherapy have remissions in more than 95% of the cases. The question arises as to whether there is a phenotype of leukemia cells that relapses and if so, what are the underlying factors. In order to analyse this, we again investigated several resistance factors mentioned above in leukemia cells of 104 children by immunocytochemical procedures. The cell samples were collected before chemotherapy, which consisted of induction therapy with prednisone, vincristine, daunomycin and L-asparaginase followed by consolidation therapy with cyclophosphamide, cytarabine, 6-mercaptopurine and intrathecal methotrexate. Of the investigated proteins, Fos, GST-pi, PKC and P-gp showed significant relationships to relapses. We performed hierarchical cluster analyses and found a cluster which was enriched with patients who relapsed and another cluster with patients who did not relapse (83).

Conclusion

Although the statistical probability of therapeutic success is known from many clinical trials, the clinical response of the individual patient still remains uncertain. Therefore, a number of test systems to detect tumor resistance against cytostatic agents have been developed over the past decades. A close inspection of the clinical correlation between the test results and the clinical data reveals that none can satisfactorily predict which drug will be most effective in the clinical setting irrespective of the fact that these tests are capable of determining the *in vitro* drug resistance with sufficient reliability. The extremely high true-negative accuracy of the tissue culture method, the clonogenic assay and the short-term test in predicting clinical drug resistance in patients with cancer indicates that all three test systems can be used to exclude antineoplastic drugs which will not be clinically useful. However, the percentages of tumors which show *in vitro* sensitivity is higher than the fraction *in vivo*.

Evidence exists that a great diversity of drug resistance mechanisms are operative in clinical drug resistance. The systematic investigation of combinations of cellular factors in cancer clearly yields improved predictive information. By using three cellular factors, the responsiveness and resistance exhibited by non-small cell lung cancer could be correctly diagnosed in about 90% of the cases.

Recently developed technologies for genome-, transcriptome- or proteome-wide analyses facilitates the simultaneous analyses of thousands of genes or proteins in a single experiment, raising expectations that it will revolutionize cancer diagnosis. On the other hand, the results of our group as well as of other authors (84) indicate that a minimal set of about 10 to 50 factors may be sufficient and may bring more robust results than sets of thousands of factors. We showed that different resistant profiles exist within tumors of homogeneous histology by means of immunohistochemistry, hierarchical cluster analyses and clustered-image maps. Thus, it is possible to identify novel subgroups of otherwise homogeneous tumor collectives. We estimate these results as one step further to the ultimate goal of prediction of drug response of each individual patient.

Since corresponding mRNA and protein expression levels are surprisingly weakly correlated to each other and quantitative mRNA data only inadequately predict the quantity of a protein in a cell (85), we suppose that protein arrays are more appropriate than mRNA micro-arrays to generate a predictive test for cancer. Among the different techniques in the field of proteomics (antibody, ligand or peptide micro-arrays, ELISA-based arrays, 2D-gel electrophoresis) we have chosen immunohistochemistry, as it is easy and convenient to perform. The combination of the recently developed tissue array technology with immunohistochemistry will speed up the sample throughput in the future by determination of the protein expression of dozens or hundreds of tumor samples on a single microscope slide (86).

In the long run, it has to be seen whether proteomics along with other "-omics" technologies will provide real hope or just another hype. It has to be taken cautiously, bearing in mind that the response to chemotherapy depends not only on intracellular, *e.g.*, molecular and biological factors, but also on extracellular factors. The relevance of pharmacokinetics and- dynamics must not be underestimated. Experience with the former predictive tests showed that additional factors can cause treatment failure and discrepancies between predictive test results and clinical response (false-positive results) *e.g.* insufficient drug dosage, inactivation of drugs by enzymes, decreased transport of the drug, or heterogeneity of the tumors. All these factors may also influence the results obtained by protein-arrays and it remains open for future discussions, as to whether the results obtained by proteomic methods are superior to those obtained by previous predictive methods. All these tests can only provide suggestions for effective therapies, but the final decision for the most beneficial treatment protocol for each patient has to be made by the clinician.

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