Protein expression profiles indicative for drug resistance of non-small cell lung cancer

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Data obtained from multiple sources indicate that no single mechanism can explain the resistance to chemotherapy exhibited by non-small cell lung carcinomas. The multi-factorial nature of drug resistance implies that the analysis of comprising expression profiles may predict drug resistance with higher accuracy than single gene or protein expression studies. Forty cellular parameters (drug resistance proteins, proliferative, apoptotic, and angiogenic factors, products of proto-oncogenes, and suppressor genes) were evaluated mainly by immunohistochemistry in specimens of primary non-small cell lung carcinoma of 94 patients and compared with the response of the tumours to doxorubicin in vitro. The protein expression profile of non-small cell lung carcinoma was determined by hierarchical cluster analysis and clustered image mapping. The cluster analysis revealed three different resistance profiles. The frequency of each profile was different (77, 14 and 9%, respectively). In the most frequent drug resistance profile, the resistance proteins P-glycoprotein/MDR1 (MDR1, ABCB1), thymidylate-synthetase, glutathione-S-transferase-π, metallothionein, O⁶-methylguanine-DNA-methyltransferase and major vault protein/lung resistance-related protein were up-regulated. Microvessel density, the angiogenic factor vascular endothelial growth factor and its receptor FLT1, and ECGF1 as well were down-regulated. In addition, the proliferative factors proliferating cell nuclear antigen and cyclin A were reduced compared to the sensitive non-small cell lung carcinoma. In this resistance profile, FOS was up-regulated and NP23 down-regulated. In the second profile, only three resistance proteins were increased (glutathione-S-transferase-π, O⁶-methylguanine-DNA-methyltransferase and major vault protein/lung resistance-related protein). The angiogenic factors were reduced. In the third profile, only five of the resistance factors were increased (MDR1, thymidylate-synthetase, glutathione-S-transferase-π, O⁶-methylguanine-DNA-methyltransferase, major vault protein/lung resistance-related protein).

Keywords: non-small cell lung carcinomas; resistance factors; hierarchical cluster analysis

Lung cancer remains a major cause of morbidity and mortality in Western countries. The majority of bronchogenic carcinomas can be classified into four histological types: small cell lung carcinomas, squamous cell lung carcinomas, adenocarcinomas, and large cell lung carcinomas. Histological features, ultra-structure, clinical course, and response to therapy indicate that small cell lung cancer is a separate entity. The other three histological subtypes are referred to as non-small cell lung cancer (NSCLC). While small cell lung carcinomas are among the most drug sensitive tumours, NSCLC are frequently resistant to drug therapy and obtaining a complete response is rare. Therefore, the drug resistance in patients receiving chemotherapy alone or when combined with radiotherapy represents a major problem in cancer treatment of patients with NSCLC.

Data obtained from multiple sources indicate that no single mechanism can explain the resistance to therapy exhibited by NSCLC. The resistance mechanisms are numerous and diverse (Valeriote and van Putten, 1975; Bradley et al., 1988; Kelley et al., 1988; Scanlon and Kashani-Sabet, 1988; Whelan et al., 1989; Huot et al., 1991; Kaino et al., 1991, Volm et al., 1991; Koomägi et al., 1995; Hickman, 1996; Volm and Mattern, 1996; Volm and Rittgen, 2000). They depend on the detoxifying capacity of the cells, repair capacity, drug delivery, cell proliferation and many other factors. Therefore, a key challenge is to determine the relative quantitative contributions of each of these mechanisms to the drug resistant phenotype. In order to understand the complex network of genes in clinically relevant drug resistance, it is rather not sufficient to investigate single genes. Holistic analyses of an entire battery of genes conferring drug resistance may be more appropriate to gain insight into the full potential of unresponsive tumours.

In an endeavor to cope with the complex resistance mechanisms, in this investigation 40 cellular parameters were evaluated in specimens of NSCLC of 94 patients. The purpose of this study was to determine the resistance profiles of NSCLC by hierarchical cluster analysis.

MATERIALS AND METHODS

Patients and tumours

Ninety-four patients (83 men, 11 women) with previously untreated NSCLC were admitted to this study. All patients were surgically treated at the Chest Hospital in Heidelberg-Rohrbach. The morphological classification of the carcinomas was conducted according to the WHO specifications. Of the carcinomas, 48 were squamous carcinomas, 34 were adenocarcinomas and 12 were large cell carcinomas. All patients were staged at the time of their surgery according to the guidelines of the American Joint Committee on
Cancer. Sixteen patients had stage I, 12 patients stage II and 66 patients had stage III tumours. The mean age of the patients was 59 years.

Detection of tumour resistance in vitro

Most of the patients were treated by surgical procedures alone. Only a small group of patients were treated by combined surgical and radiation treatment or chemotherapy but the additional radiation treatment and chemotherapy had no significant effect on patient survival time (P > 0.1) and on the cluster analysis. For determining the resistance of tumours we used a short-term in vitro test that has been described previously (Volm et al, 1979, 1988a). Its basic feature is measurement of changes in the incorporation of radioactive nucleic acid precursors into tumour cells after addition of doxorubicin. We found that anthracyclines (e.g. doxorubicin) can be used as reference compound for multiple resistance. Tumours were defined as being sensitive or resistant depending on whether nucleotide uptake was inhibited by more or less than 65% at a concentration of doxorubicin of 10 μg ml⁻¹. This threshold was based on prior clinical correlation (Volm et al, 1979).

Immunohistochemistry

The previously described method was used to detect the proteins in paraffin-embedded specimens (Volm et al, 1991, 1998; Koomägi and Volm, 1999). Briefly, formalin-fixed and paraffin-embedded tissue were deparaffinised. After pre-incubation with hydrogen peroxide and protein blocking solution, the primary antibodies were applied for 16 h at 4°C. After incubation with secondary antibodies, the streptavidin biotinylated peroxidase complex was added and the peroxidase activity visualised with amino-9-ethylcarbazole. Counter-staining was performed with haematoxylin. Both negative and positive controls were conducted. Negative controls were prepared by omitting the primary antibodies and by substituting irrelevant antibodies for the primary antibodies. The specificity of the reactions were proved by Western blots. Three observers independently evaluated the results from the immunohistochemical staining without having any prior knowledge of an individual patient’s clinical data. The evaluated agreement in 90–95% of the samples. The other specimens (5–10%) were re-evaluated and then classified according to the classification most frequently given by the observers. To evaluate the protein expression, the staining intensity or the staining intensity and the percentages of positive cells were determined.

A part of the antibodies for the detection of resistance-proteins were gifts from several laboratories, others were commercially available. The appropriate concentrations were found by additional experiments. Anti-glutathione S-transferase-π (GSTP1) was kindly donated by Dr K Satoh (University School of Medicine, Hiroasaki, Japan) anti-DNA topoisomerase II (TOP2A) by Dr L Liu (John Hopkins Oncology Center, Baltimore, MD, USA); anti-metallothionein (MT) by Dr PC Huang (Johns Hopkins University, Baltimore, MD, USA); anti-thymidylate synthetase (TYMS) by Dr B Yates (Burroughs Welcome, Research Triangle Park, Cornelius USA); and anti-O'-methylguanine-DNA-methyl-transferase (MGMT) by Dr B Li (University Singapore, Singapore). The anti-P-glycoprotein (MDR1, ABCB1) antibody JSB-1 was obtained from Sanbio (Uden, Netherlands); anti-catalase (CAT) from Calbiochem (La Jolla, CA, USA); anti-heat shock protein 70 (HSP70A1) from Dako Diagnostic (Hamburg, Germany), and anti-major vault protein/lung resistance-related protein (MVP/LRP) from Dunn Labotechnik (Asbach, Germany). For detection of the proliferative activity, anti-cyclin A (CCNA), anti-CDK2 and anti-CDK4 were obtained from Santa Cruz Biotechnology (Heidelberg, Germany). Anti-cyclin D1 (CCND) was from Calbiochem/Novabiochem (Baden-Soden, Germany) and PCNA from Dianova (Hamburg, Germany). The antibody for detection of the apoptotic factor FAS/CD95 (TNFSF6) was from Immunotech (Hamburg, Germany) and anti-FAS ligand (TNFSF6) and anti-caspase 3 (CASP3) from Santa Cruz Biotechnology. The antibodies for staining of angiogenic factors were anti-VEGF obtained from Dianova, anti-tissue factor (TF, F3) from Biodesign (Kennebunk, MA, USA), anti-bFGF, anti-FGFR and anti-FLT1 from Santa Cruz Biotechnology.

Measurement of micro-vessel density

Blood vessels were highlighted by staining endothelial cells for factor VIII using the streptavidin-biotin peroxidase complex method. Micro-vessel density was determined by counting labelled capillaries in the areas of highest vascularisation within the tumour mass. Individual counts of blood vessels were determined by light microscopy in a 250× field as described earlier (Weidner et al, 1991).

Assessment of apoptosis

Apoptotic cell death was detected with a non-radioactive 3'-end DNA labelling technique which used the in situ cell death detection kit (TUNEL reaction). The procedure was described earlier (Stammuner and Volm, 1996).

DNA cell cycle analysis by flow cytometry

A mixture of propidium iodide and 4′-6-diamidino-2-phenylindole was applied simultaneously with RNAse after methanol fixation and protease digestion of single cell suspensions (Volm et al, 1988b). Flow cytometry analysis was undertaken with an ICP-22 (Phywe, Göttingen, Germany). Peripheral blood leukocytes from healthy donors were used as a calibration standard for DNA diploidy. Parallel measurements, both including and omitting the standard, were performed. The cell cycle analysis was performed using integrated Gaussian fittings. A computerised subtraction of exponentially decreasing corrections beginning with the peak of the first standard, were performed. The cell cycle analysis was omitted in cases that exhibited interspersed cell populations.

Statistical analysis

The doxorubicin resistance parameter (short-term test) is expressed as a percentage of the controls. These values define a ‘sensitive’ group (less than 65% at a concentration of 10⁻² mg ml⁻¹ doxorubicin) and a ‘resistant’ group (not less than 65%). This classification is used later on to examine whether other parameters show significant relationships.

The immunohistochemical parameters were evaluated on either a binary scale (‘no reaction’ or ‘reaction’, coded as ‘−’ or ‘+’) or an ordinal scale ‘no reaction’, ‘weak’, ‘moderate’ or ‘strong reaction’ (coded as ‘−’, ‘+’, ‘++’ or ‘+++’). The apoptotic indices,
micro-vessel density and the cell cycle phases were counting variables and, therefore, the median values were used. To find out the predictive value of these parameters with respect to doxorubicin resistance Fisher’s exact tests were performed. The results of these tests with P-values <0.1 are summarised in Table 1. The parameters with a P value greater than 0.1 were not analysed any further.

Hierarchical cluster analysis is an explorative statistical method and aims to group at first sight heterogeneous objects into clusters of homogeneous objects. Objects are classified by calculation of distances according to the closeness of-between-individual distances. All objects are assembled into a cluster tree (dendrogram). Thus, objects with tightly related features appear together, while the separation in the cluster tree increases with progressive dissimilarity. Cluster analyses applying average- or complete-linkage methods were done by means of the WinSTAT program (Kalma Company). Missing values are automatically omitted by the program and the closeness of two joined objects was calculated by the number of data points they contained. In order to calculate distances of all variables included in the analysis, the program automatically standardises the variables by transforming the data with a mean=0 and a variance=1. To construct clustered-image maps (CIM), two dendrograms were related to each other. The 94 NSCLC were cluster-ordered on the basis of their expression pattern across the resistance factors. Tumours with most identical expression patterns were placed closer to each other. The position of the object along the x-ordinate. The 94 NSCLC were cluster-ordered across the resistance factors. Tumours with most identical expression patterns of resistance factors appear side by side on the y-ordinate.

RESULTS

The objective of this investigation was to evaluate the profile of protein expression involved in resistant NSCLC. For this reason, the expressions of 40 factors in primary NSCLC of 94 patients were determined and correlated with the data obtained by the in vitro resistance test. Examples are given in Figure 1. Of the 94 patients with NSCLC 70 patients had resistant carcinomas, whereas 24 patients revealed sensitive carcinomas. As visible in Figure 1, P-glycoprotein is positively correlated with resistance while VEGF is negatively correlated.

In a first step, we analysed the relationships of the expression levels of the 40 factors with sensitivity/resistance by Fisher’s exact tests. Of the investigated resistance proteins P-glycoprotein (MDR1, ABCB1), glutathione-S-transferase-π (GSTP1), metallothionein (MT), Oβ-methylguanine-DNA-methyltransferase (MGMT), lung resistance-related protein (MVP/LRP), thymidylate synthetase (TYMS), DNA-topoisomerase II (TOPII), catalase (CAT) and heat shock protein 70 (HSP 70) six proteins (MDR1, TYMS, GSTP1, MT, MGMT and MVP/LRP) showed a relationship to sensitivity/resistance (P<0.1) (Table 1). These six proteins were increased in resistant NSCLC. Of the proliferative factors analysed (PCNA, cyclin A, cyclin D, the cyclin-dependent kinases cdk2 and cdk4, cell cycle phases) only PCNA and cyclin A (CCNA) exhibited a relationship to sensitivity/resistance and were down-regulated in resistant carcinomas. Of the angiogenic proteins tested, vascular endothelial growth factor (VEGF), its receptor FLT1 and platelet-derived endothelial growth factor (ECGF1) showed a relationship to sensitivity/resistance. These factors were reduced in resistant carcinomas. This was confirmed by the reduction of micro-vessel density in resistant tumours (Table 1). Such relationship was not found with basic fibroblast growth factor (bFGF), its receptor FGFR and with angiotatin. No relationship exists between the expression of tissue factor and the resistance of tumours. Of the pro-apoptotic factors investigated FAS/CDD95, FAS ligand, and caspase-3 only FAS/CDD95 was significantly reduced in resistant NSCLC. The anti-apoptotic factor BCL-2 showed no obvious relationship to sensitivity/resistance of NSCLC. There is huge evidence that proto-oncogenes and suppressor genes are implicated in resistance of tumours. Therefore, we investigated the products of c-fos, c-jun, c-myc, c-erbB1, c-erbB2, c-K-ras, c-H-ras, c-N-ras, r-b, p53, nm23 and p16%. Of these proteins only FOS, ERBB2, and NM23 revealed a correlation to sensitivity/resistance of NSCLC. Finally, hypoxia causes a wide range of responses in tumours. Therefore, we assessed whether hypoxia-inducible factors (HIF) may also play a role in the resistance of NSCLC. Both HIF-1α and HIF-1β did not reveal a relationship to sensitivity/resistance of NSCLC. In Table 1, the 16 factors are ordered which showed relationships to the doxorubicin response of NSCLC (P<0.1) The parameters with P-values greater than 0.1 were not analysed any further.

This kind of analysis, however, does not allow an insight to complex expression profiles which constitute resistant or sensitive tumour phenotypes. Therefore, we decided to perform hierarchical cluster analyses which may be more suited to unravel the full potential of such data sets for an integrated understanding of drug resistance. We subjected these 16 proteins to hierarchical cluster analyses (Figure 2, dendrogram on the right). We divided the dendrogram into five clusters and correlated them with the in vitro resistance data which were included as parameter into the cluster analysis (Table 2). Sensitive and resistant NSCLC were separated in the clusters (P=7.58 E−7). Cluster 1 (n=23 cases) is enriched with sensitive carcinomas (78%), while cluster 2 (n=6 cases), cluster 4 (n=53 cases) and cluster 5 (n=10 cases) were enriched with resistant carcinomas (83, 89 and 90% respectively). These clusters showed no relationships to other clinical parameters (stage, survival).

The mean values of the 16 parameters of all investigated carcinomas of the clusters were measured and the ratios of resistant/
sensitive clusters determined (Table 3). The cluster analysis revealed that three different resistance profiles exist. The frequency of the resistance profiles are different (cluster 4: 77%, cluster 5: 14%, cluster 2: 9%).

In the most frequent drug resistance profile (cluster 4) all resistance proteins investigated (MDR1, TYMS, GSTP1, MT, MGMT, MVP/LRP) were up-regulated. Micro-vessel density and the angiogenic factors ECGF1, VEGF, FLT1 were down-regulated. Additionally, the proliferative factors PCNA and CCNA were reduced. The apoptotic factor FAS/CD95 was less expressed than in sensitive carcinomas.

Table 2 Separation of five NSCLC clusters obtained by the hierarchical cluster analysis shown in Figure 2 (right side) and comparison to chemosensitivity. The distribution of sensitive and resistant NSCLC was determined with the in vitro short-term test. Cut-off of the short-term test: 65%.

| Cluster | 1 | 2 | 3 | 4 | 5 |
|---------|---|---|---|---|---|
| Sensitive | 16 | 1 | 1 | 5 | 1 |
| Resistant | 7 | 5 | 1 | 48 | 9 |

$P=7.58 \times 10^{-7}$ (Fisher exact test).

Figure 1 Relationship between the drug response (doxorubicin) as determined by the in vitro short term test (ordinate) and the immunohistochemical reaction of P-glycoprotein/MDR1 and VEGF. The intensity of immunostaining (negative, weak, moderate, high) is specified as –, +, ++, +++.

Figure 2 Dendrograms and clustered image map obtained by hierarchical cluster analysis (complete linkage method). The dendrogram on the right shows the clustering of 94 NSCLC and the dendrogram on the top of 16 resistance factors. Light fields indicate ‘not expressed’ and dark fields indicate ‘expressed’ according to the partition for each resistance factor shown in Table 2. Missing values are depicted in white.
DISCUSSION

The advances in molecular and cellular biology have opened new avenues for the characterisation of drug resistant tumours. Protein and RNA expression are in the centre of interest. Protein detection by immunohistochemistry and RNA detection by Northern blotting or reverse transcription-polymerase chain reaction (RT–PCR) may be more suited for clinical application than assays such as Western blotting or RNase protection assay. In this study, we used mainly immunohistochemical assays to analyse expression profiles of proteins of NSCLC.

To analyse the effect of doxorubicin, we used the nucleotide incorporation test (in vitro short-term test), because the patients were operated only. Previously, different in vitro test systems (tissue culture assay, colony assay and short term test) to monitor the resistance were compared and similar results with all three methods were obtained (Volm et al, 1987). In a clinical study, 55 of 57 tumours that were resistant in the short-term test were also clinically progressive (Group for Sensitivity Testing of Tumors (KSS), 1981). Several authors have confirmed this consistency between in vitro test results and the clinical results (Auner et al, 1989; Khoo et al, 1989). These evidences let us believe that the in vitro short term is suitable to determine the resistance of NSCLC.

There is striking evidence that a wide variety of drug resistance mechanisms are operational in clinically relevant drug resistance. In this analysis, several resistance proteins and additional resistance-related factors were changed in resistant NSCLC in comparison to sensitive carcinomas. The reason for this concomitant expression of different factors may be the result of inducing a cascade of resistance-related gene product which can be triggered by environmental factors such as smoking (Volm et al, 1981, 1999; Mattern et al, 1998). Another possible explanation is that lung cancer is mostly detected at a relatively late stage. These carcinomas are mainly hypoxic and the vascular network that supplies oxygen and nutrients is substantially lower. In fact, in our study the micro-vessel density and several angiogenic factors were reduced in resistant NSCLC.

Data obtained in a variety of systems show that the proliferation may also be an important resistance factor (Kohn et al, 1994; Cordon-Cardo, 1995). Indeed, we found in the present study that the proliferative factors were decreased in resistant NSCLC. Apoptosis or programmed cell death is an important homeostatic mechanism that balances cell production with cell death (Thompson, 1995). In our analysis, FAS/C95 was also lower in resistant NSCLC compared to sensitive carcinomas.

Because indications exist that proto-oncogenes and tumour suppressor genes may be implicated in tumour resistance (Burt et al, 1988), we examined the expressions of the proteins of some of these factors. In our analysis, the expressions of ERBB2, NM23, and FOS played an important role in resistant NSCLC. ErB2 encodes a protein that acts as a transmembrane growth factor receptor and showed a similar reaction as the proliferative factors CCNA and PCNA on the response. NM23 which is a putative metastasis suppressor gene has also been shown to be associated with cell proliferation and apoptosis (Venturelli et al, 1995). FOS and JUN constitute the AP-1 protein complex which binds specifically to the AP-1 binding site in promotors of resistance genes (Teeter et al, 1991). Though this is known for a decade, the full relevance of AP1 and FOS may have been underestimated as of yet.

The multi-faceted nature of drug resistance implies that the analysis of entire expression profiles may predict drug resistance with higher accuracy and may be superior to single gene expression studies. By means of clustered image mapping (CIM) we found four different protein expression profiles. One expression profile was characterised by low expression of drug resistance proteins and high expression of proliferation/angiogenesis-regulating proteins (CIM areas M to O). This expression profile was indica-

**Table 3** Ratio (resistant/sensitive) of the parameters in different clusters obtained in the dendrogram shown in Figure 1 (right side)

| Parameter | Cluster 4/1 | Ratio | Cluster 5/1 | Ratio | Cluster 2/1 | Ratio |
|-----------|-------------|-------|-------------|-------|-------------|-------|
| MDR1 (ABCB1) | 2.0 | 1.0 | 2.2 |
| TYMS | 2.3 | 0.8 | 2.6 |
| GSTP1 | 2.0 | 1.8 | 2.7 |
| MT | 1.8 | 0.8 | 1.1 |
| MGMT | 2.5 | 3.4 | 3.6 |
| MVP/LRP | 12.5 | 8.0 | 26.0 |
| ECGF1 | 0.4 | 0.1 | 0.8 |
| NPI | 0.1 | 1.1 | 1.5 |
| Micro-vessel density | 0.6 | 0.6 | 1.0 |
| VEGF | 0.7 | 0.1 | 0.9 |
| FAS (CD95, TNFRSF6) | 0.6 | 0.7 | 1.4 |
| CCNA | 0.6 | 0.7 | 1.0 |
| ERBB2 | 0.5 | 0.4 | 1.6 |
| FLT1 | 0.6 | 0.6 | 1.0 |
| PCNA | 0.4 | 0.1 | 1.4 |
| FOS | 1.7 | 1.5 | 0.9 |

Abbreviations: see legend to Table 1.

Of the proto-oncogenes and suppressor genes FOS was up-regulated, while NM23 and ERBB2 were down-regulated.

The analysis of the carcinomas of cluster 5 shows that only three of the six investigated resistance proteins were up-regulated (GSTP1, MGMT, MVP/LRP). Again, micro-vessel density was reduced and the angiogenic factors (VEGF, ECGF1) were more down-regulated than those of the carcinomas in cluster 4. The proliferative and apoptotic factors were reduced. In contrast to the carcinomas in cluster 4, NM23 was not changed.

The evidence of the third resistance profile (cluster 2) is limited because of the small number of carcinomas in this cluster. Only five resistance proteins (MDR1, TYMS, GSTP1, MGMT, MVP/LRP) were increased in comparison to sensitive tumours, while the other resistance factors revealed only marginal changes.

Next, we performed a cluster analysis using the 16 resistance factors and found a dendrogram which can be separated into three areas (Figure 2, top). The first area contained three parameters (VEGF, MGMT and GSTP1). The second area contained in their majority proliferation- or angiogenesis-regulating proteins (e.g. ERBB2, PCNA, ECGF1, FLT1, and CCNA). In the third area were genes known to contribute to drug resistance and apoptosis (FAS/CD95, MT, TYMS, FOS, MDR1).

As shown in Figure 2, the CIM can be subdivided into several areas (A to O). The sensitive NSCLC (CIM areas M and O) were characterised by a less frequent expression of drug resistance proteins than resistant tumours of CIM areas D and F. On the other hand, these resistant tumours revealed a lower expression of proliferation/angiogenesis-regulating proteins (CIM area E) compared to sensitive tumours (CIM area N). The tumours grouped in CIM areas A to C were resistant as well, but they showed a different protein expression profile compared to the resistant tumours of CIM areas D to F. These NSCLC exhibited a rather low expression of drug resistance genes (except MGMT and GSTP1; CIM area A) and proliferation/angiogenesis-regulating proteins (CIM areas B and C). Resistant tumours of CIM areas J to L consisted of only few cases, but they seem to be distinct from the other resistant tumours of CIM areas A to I with a strong expression of all proteins investigated. Cluster 3 consisted of only two tumours and are thus less informative (CIM areas G to I). The clustering was independent of stage of tumours (P>0.9) and of survival of patients (P>0.7).
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These results may have important implications for diagnosis and therapy of NSCLC. Our data envision the definition of characteristic resistance gene profile able to predict clinical response to chemotherapy. Although the statistical probability of the resistance profile of NSCLC may be known, the clinical response of the individual patient remains still uncertain. However, the recently thriving micro-array technology may allow simultaneous analyses of thousands of genes of a single patient in a single experiment (Shim et al, 1998; Golup et al, 1999; Sgroi et al, 1999; Ono et al, 2000; Ross et al, 2000; Scherf et al, 2000). The idea to generate a predictive test for lung cancer patients is gaining ground with such novel approaches. This concept embodies to develop individual cytostatic treatment schedules for each patient. On the other hand, it has been suggested that a minimal set of about 10 to 50 genes may provide more robust results than sets of thousands of genes (Wooster, 2000). It is still open to discussion, whether results obtained from large-scale gene expression experiments may be of limited utility for routine diagnostics and whether the wealth of genes not involved in resistance produce a high background which masks the view of the relevant genes. The resistance proteins and the resistance-related factors which we found in the present analysis may be suitable parameters to predict treatment response of NSCLC. We believe that these parameters together with hierarchical cluster analysis as appropriate tool to identify responding or non-responding tumours are determinants which will ease the development of a predictive test methodology for NSCLC.

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