Expression Profile of Genes in Non-Small Cell Lung Carcinomas from Long-Term Surviving Patients

Manfred Volm, Reet Koomägi, Jürgen Mattern, and Thomas Effert

German Cancer Research Center, D-69120 Heidelberg, Germany [M. V., R. K., J. M.], and Virtual Campus Rhineland-Palatinate, D-55033 Mainz, Germany [T. E.]

ABSTRACT

Purpose: Non-small cell lung cancer (NSCLC) is usually associated with a poor prognosis. Some patients survive their disease, and the underlying molecular mechanisms are still poorly understood. The purpose of this investigation was to evaluate expression profiles of proteins determining the survival of NSCLC patients for 5 years.

Experimental Design: The expression of 21 gene products was evaluated immunohistochemically in paraffin-embedded primary NSCLCs from 216 patients. The data were correlated with the survival times of the patients (survival of more or less than 5 years) by means of χ² test and hierarchical cluster analysis.

Results: The relationships of patients' survival and 21 parameters were determined including oncogene and tumor suppressor products and proliferative, apoptotic, and angiogenic factors. FOS, F53, RAS, ERBB1, JUN, PCNA, cyclin A, FAS/CD95, and HIF-1ß revealed a correlation to survival. In a second step, these nine parameters were further analyzed by hierarchical cluster analyses of all patients, of stage III patients, and of patients with squamous cell lung carcinomas. We identified clusters with significantly more long-term survivors. The expression of FOS, JUN, ERBB1, and cyclin A or PCNA were decreased in carcinomas of patients with long-term survival.

Conclusions: The expression profile of these factors predicts a significantly better long-term outcome of NSCLC patients. This may have implications for the development of individualized therapy options in the future.

INTRODUCTION

Lung cancer is the major cause of cancer-related death in the Western hemisphere (1). The majority of bronchogenic carcinomas can be histologically classified into four types: small cell lung carcinomas, adenocarcinomas, squamous cell lung carcinomas, and large cell carcinomas. The histological features, clinical course, and response to therapy indicate that small cell lung carcinomas are a separate entity, whereas the behavior of the other three histological subtypes is similar. Therefore, these are combined within the larger group of NSCLCs.² NSCLCs represent 75–80% of all cases of lung cancer and are usually associated with a poor prognosis. Although much is known about predisposing factors, natural history, and the outcome of NSCLC, our understanding is still incomplete. However, the advances in molecular and cellular biology have opened new avenues for the characterization of these tumors.

The purpose of this study was to determine the protein expression profile of long-term survivors having NSCLC (survival times of >5 years). For this reason, we analyzed 21 parameters including proliferative, apoptotic, and angiogenic factors, proto-oncogenes, and tumor suppressors by immunohistochemistry.

Cyclins and cdks are universal regulators of cellular proliferation in eukaryotic cells. Several classes of cyclins are up- and down-regulated at specific points during the cell cycle in a wave-like manner (2). The complex formed by cyclin D1 and cdk4 governs G₁ progression, whereas cyclin A together with cdk2 regulate entry into and progression through the S-phase. The PCNA is involved in the DNA synthesis. Therefore, we analyzed the influence of cyclin A and cyclin D, the cdks cdk2 and cdk4, and PCNA on the outcome of patients.

Cell growth in general is governed by a complex molecular network. Other genes not directly involved in cell cycle progression may also contribute to tumor growth and outcome of patients. Apoptosis or programmed cell death is a crucial mechanism of cellular homeostasis in organisms (3, 4). In this investigation, we analyzed the influence of the proapoptotic factor Fas/CD95 on the survival of the patients.

Angiogenesis, the development and formation of new blood vessels, is important in a variety of processes including tumor growth (5). The VEGF and the fibroblast growth factor are angiogenic molecules that might influence the outcome of the patients. PD-ECGF and TF are also involved in angiogenesis and tumor growth. In the present study, therefore, we analyzed the relationships between VEGF, bFGF, PD-ECGF, TF, and the survival of patients.

There is much evidence that proto-oncogenes and tumor suppressor genes are implicated in the growth of tumors by acting as growth factors or their receptors (i.e., c-ERB-B1 and c-ERB-B2), signal transducers (i.e., RAS), or transcription fac-

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² The abbreviations used are: NSCLC, non-small cell lung carcinoma; PCNA, proliferating cell nuclear antigen; cdk, cyclin-dependent kinase; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; PD-ECGF, platelet-derived endothelial cell growth factor; TF, tissue factor; HIF, hypoxia-inducible factor.
tors (i.e., c-FOS, c-JUN, and c-MYC; Refs. 6–8). Tumor suppressors such as p53 guide cellular integrity by regulating cell cycling and arrest, DNA repair, and apoptosis. For this reason, we determined the influence of FOS, JUN, MYC, ERBB1, ERBB2, K-RAS, H-RAS, N-RAS, and P53 on the survival of patients.

Hypoxia causes a wide range of responses in tumors, and HIFs (HIF-1α) may also play a role in survival (9, 10). Therefore, we assessed whether HIF-1α and HIF-1β have an influence on the outcome of patients with NSCLC.

Thus, we analyzed both the statistical significance of the expression of 21 different proteins in primary NSCLC of 216 patients and their relationships to the outcome of the patients (survival of less or more than 5 years). Of these parameters, those with a significant interrelationship ($P < 0.1$) were subsequently subjected to hierarchical cluster analysis with the aim to define expression profiles explaining the longer survival times of patients with NSCLC.

**MATERIALS AND METHODS**

**Patients.** Two hundred and sixteen consecutive patients with previously untreated NSCLCs were admitted to this study. All patients were operated at the Chest Hospital in Heidelberg-Rohrbach. The morphological classification of the carcinomas was conducted according to the WHO specifications: 123 (57%) were squamous carcinomas, 59 (27%) were adenocarcinomas, and 34 (16%) were large cell carcinomas. All patients were staged at the time of their surgery following the guidelines of the American Joint Committee on Cancer Staging and End Result Reporting. Forty-two patients (19%) had stage I tumors, 17 (8%) patients had stage II tumors, and 157 (73%) had stage IIIA tumors. The average age of the patients was 57 years. One hundred and forty-seven patients were treated only by surgical procedures, 29 patients were additionally treated with antineoplastic drugs, and 41 patients were treated with irradiation. The additional radiation treatment and chemotherapy had no significant effect on patient survival time. Follow-up data were obtained without having any prior knowledge of an individual patient’s clinical data. The immunohistochemical parameters were evaluated on either binary scales (no reaction, 0; reaction, 1; coded as $-$ or $+$, respectively) or ordinal scales (no reaction, 0; weak, 1; moderate, 2; strong reaction, 3; coded as $-$, $+$, $++$, and $++++$, respectively). The evaluations agreed 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.

**Antibodies.** Proliferative activity was determined using anti-cyclin A (clone H-432; dilution, 1:50), anti-cdk2 (clone M2; dilution, 1:200), and anti-cdk4 (clone C22; dilution, 1:100; all obtained from Santa Cruz Biotechnology, Heidelberg, Germany). Anti-cyclin D1 (clone Ab-3; dilution, 1:10) was from Calbiochem-Novabiochem (Baden-Soden, Germany) and PCNA (clone PC10; dilution, 1:10) was from Dianova (Hamburg, Germany). The antibody for detection of the apoptotic factor Fas/CD95 was from Immunotech (Hamburg, Germany; clone UB-2; dilution, 1:100).

The antibodies for staining of angiogenic factors were anti-VEGF (clone Ab-2; dilution, 1:10) obtained from Dianova, anti-TF (clone TFB; dilution, 1:50) from Biodesign (Kennebunk, MA), and anti-bFGF (clone 147; dilution, 1:200) from Santa Cruz Biotechnology. Anti-PD-ECGF (clone 654-1; dilution, 1:50) was a generous gift from Dr. Tanaka (Nippon Roche Research Center, Kamakura, Japan).

For the detection of the proto-oncogene and suppressor gene products, we used the following antibodies: P53 (clone Pab 1801), c-FOS (clone Ab-2), c-JUN (clone c-Jun/AP-1), c-MYC (clone Ab-3), ERBB-1 (clone Ab-4), ERBB-2 (clone Ab-3), c-N-RAS (clone F155–277), c-H-RAS (clone 235–1.7), and c-K-RAS (clone 234–4.2). All antibodies for these proto-oncogene and suppressor gene proteins were purchased from Novus Biologicals (Littleton, CO) and were applied at a concentration of 10 µg/ml.

To detect HIF-1α, the monoclonal mouse anti-HIF-1 (HIF-1α, clone HIF-1α 67; dilution, 1:1000) and the polyclonal rabbit anti-HIF-1β (HIF-1β/ARNT; dilution, 1:1700) obtained from Novus Biologicals (Littleton, CO) were used.

**Assessment of Expression.** Three observers independently evaluated the results from the immunohistochemical staining without having any prior knowledge of an individual patient’s clinical data. The immunohistochemical parameters were evaluated on either binary scales (no reaction, 0; reaction, 1; coded as $-$ or $+$, respectively) or ordinal scales (no reaction, 0; weak, 1; moderate, 2; strong reaction, 3; coded as $-$, $+$, $++$, and $++++$, respectively). The evaluations agreed 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.

**Statistical Analysis.** To find out the relationships of the parameters with respect to survival times (less or more than 5 years), we used χ² tests. Parameters with Ps $>$0.1 were not analyzed any further. Then, we performed hierarchical cluster analysis, which is an explorative statistical method that groups heterogeneous objects into clusters of homogeneous objects. All objects are assembled into a cluster tree (dendrogram). Thus, objects with tightly related features appear together, whereas the separation in the cluster tree increases with progressive dissimilarity. Of the 21 parameters, 9 were analyzed by hierarchical cluster analysis which showed a relationship to survival ($P < 0.1$). Cluster analyses applying the complete-linkage method were done by means of the WinSTAT program (Kalmia 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. To calculate distances of all variables included in the analysis, the program automatically standardizes the variables by transforming the data with a mean = 0 and a variance = 1. The clusters...
RESULTS

The purpose of this investigation was to evaluate profiles of protein expression involved in NSCLC of patients who survived 5 years after operation. For this reason, the expressions of 21 gene products in primary human NSCLCs of 216 patients were analyzed, and the proteins were correlated with the survival times. Forty-nine patients (23%) survived 5 years.

In a first step, we analyzed the relationships of the 21 parameters with the survival times (less or more than 5 years) of the patients. Of the proto-oncogene and suppressor gene products, FOS (P < 0.0001), PS3 (P < 0.01), N-RAS (P = 0.02), and ERBB1 (P < 0.02) revealed a significant relationship to survival, whereas JUN showed only a marginal correlation (P = 0.08; Table 1). We did not find a correlation between MYC (P > 0.6), ERBB2 (P > 0.1), K-RAS (P > 0.2), H-RAS (P > 0.3), and the survival of patients with NSCLC.

Of the analyzed proliferative factors, PCNA (P < 0.04) and cyclin A (P < 0.03) showed a significant correlation to survival (Table 1), whereas cyclin D (P > 0.8), cdk2 (P > 0.5), and cdk4 (P > 0.3) did not. There was also a significant relationship between FAS/CD95 expression and survival (P < 0.04; Table 1).

A significant influence of the angiogenic factors on survival was not detected (VEGF, P > 0.9; bFGF, P > 0.5; PD-ECGF, P > 0.7). Similarly, TF did not reveal a relationship to survival (P > 0.3).

We found a significant correlation with HIF-1β (P < 0.03; Table 1) but not with HIF-1α (P > 0.9). Thus, 9 of 21 parameters analyzed showed a relationship to the survival of patients with a cutoff of 5 years. The expressions levels of FOS, N-RAS, ERBB1, JUN, cyclin A, and PCNA were reduced, whereas pS3, HIF-1β, and FAS/CD95 were increased in carcinomas of long-term survivors. Multivariate analyses showed that ERBB1 and FOS are indeed independent prognostic factors for survival in addition to stage and histology (stage, P < 0.005; histology, P = 0.018; FOS, P = 0.007; stage, P = 0.003; histology; P = 0.016; ERBB1, P = 0.041).

Table 1 Correlation between the survival of patients with NSCLC (<5/>5 years) and cellular parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Partitiona</th>
<th>All NSCLC (%)b</th>
<th>P</th>
<th>Stage IIIA (%)b</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOS</td>
<td>- vs. +, +++, +++</td>
<td>40 vs. 13</td>
<td>&lt;0.0001</td>
<td>34 vs. 13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PS3</td>
<td>- vs. +, +++, +++</td>
<td>16 vs. 32</td>
<td>&lt;0.01</td>
<td>11 vs. 26</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>N-RAS</td>
<td>- vs. +, +++, +++</td>
<td>37 vs. 19</td>
<td>&lt;0.02</td>
<td>34 vs. 12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ERBB1</td>
<td>- vs. +, +++, +++</td>
<td>35 vs. 20</td>
<td>&lt;0.02</td>
<td>27 vs. 15</td>
<td>0.11</td>
</tr>
<tr>
<td>Cyclin A</td>
<td>- vs. +, +++, +++</td>
<td>30 vs. 17</td>
<td>&lt;0.03</td>
<td>21 vs. 15</td>
<td>0.48</td>
</tr>
<tr>
<td>HIF-1β</td>
<td>- vs. +, +++, +++</td>
<td>14 vs. 24</td>
<td>&lt;0.03</td>
<td>8 vs. 26</td>
<td>0.06</td>
</tr>
<tr>
<td>FAS/CD95</td>
<td>- vs. +</td>
<td>16 vs. 32</td>
<td>&lt;0.04</td>
<td>12 vs. 29</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>PCNA</td>
<td>- vs. +, +++, +++</td>
<td>28 vs. 13</td>
<td>&lt;0.04</td>
<td>20 vs. 13</td>
<td>0.22</td>
</tr>
<tr>
<td>JUN</td>
<td>- vs. +, +++, +++</td>
<td>30 vs. 20</td>
<td>0.08</td>
<td>25 vs. 12</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

a Partition of “negative” and “positive” classified immunostaining. The most appropriate concentrations of the antibodies were determined in preliminary experiments.

b Frequency (%) of long-term survivors corresponding to the partition.

Chi squares (χ²) test.

Stage and histology are well-known prognostic factors for survival. To exclude the influence of these prognostic factors into the analysis, we further analyzed only stage IIIA carcinomas and squamous cell lung carcinomas separately. With these more homogeneous groups of patients, we obtained similar results (data of stage IIIA carcinomas are given in Table 1).

In a second step, we analyzed these 9 parameters, which revealed a correlation to survival by hierarchical cluster analysis, to find out a protein expression profile for long-term survivors. The dendrogram of the cluster analysis of all patients is given in Fig. 1. The resulting clusters were correlated with the patients’ survival (cutoff 5 years; Table 2). Clusters 1 and 3 are enriched with patients who survived 5 years. In Table 3, the mean values of immunohistochemical staining with 95% confidence intervals are given for the carcinomas of all patients and for those of clusters 1 and 3. The expression levels of FOS, PCNA, ERBB1, and N-RAS are significantly different in clusters 1 and 3 versus all cases (confidence intervals were not overlapping; α = 0.05).

The more homogeneous groups of patients (stage IIIA, squamous cell lung carcinomas) were investigated by cluster analyses. Most of the long-term survivors with stage IIIA were
Table 2  Separation of clusters of all NSCLC (n = 216) obtained by the hierarchical cluster analysis shown in Fig. 1 and comparison with survival times (<5/>5 years)

<table>
<thead>
<tr>
<th>Cluster</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5 yr</td>
<td>38</td>
<td>11</td>
<td>11</td>
<td>46</td>
<td>17</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>&gt;5 yr</td>
<td>17</td>
<td>0</td>
<td>11</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

* P = 0.004.

Table 3  Mean values of immunohistochemical staining with confidence intervals (α = 0.05) of all cases (n = 216) and of the cases of clusters 1 and 3 (enriched with long-term survivors; n = 77)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>All cases</th>
<th>Clusters 1 and 3</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOS</td>
<td>1.03 (0.89–1.16)</td>
<td>0.53 (0.36–0.70)</td>
<td>−1.9</td>
</tr>
<tr>
<td>PCNA</td>
<td>0.86 (0.69–1.02)</td>
<td>0.46 (0.25–0.66)</td>
<td>−1.9</td>
</tr>
<tr>
<td>ERBB1</td>
<td>1.69 (1.56–1.83)</td>
<td>1.08 (0.87–1.30)</td>
<td>−1.6</td>
</tr>
<tr>
<td>N-RAS</td>
<td>1.55 (1.39–1.71)</td>
<td>1.00 (0.77–1.23)</td>
<td>−1.6</td>
</tr>
<tr>
<td>PS3</td>
<td>0.99 (0.85–1.13)</td>
<td>0.77 (0.55–1.00)</td>
<td>−1.3</td>
</tr>
<tr>
<td>JUN</td>
<td>0.91 (0.77–1.04)</td>
<td>0.76 (0.57–0.95)</td>
<td>−1.2</td>
</tr>
<tr>
<td>Cyclin A</td>
<td>1.62 (1.46–1.78)</td>
<td>1.68 (1.40–1.96)</td>
<td>1.0</td>
</tr>
<tr>
<td>FAS/CD95</td>
<td>0.54 (0.46–0.61)</td>
<td>0.54 (0.41–0.67)</td>
<td>1.0</td>
</tr>
<tr>
<td>HIF-1β</td>
<td>2.27 (2.12–2.42)</td>
<td>2.24 (1.99–2.48)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 4  Separation of clusters of stage IIIA-NSCLC (n = 157) obtained by hierarchical cluster analysis and comparison with survival times (<5/>5 years)

<table>
<thead>
<tr>
<th>Cluster</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5 yr</td>
<td>31</td>
<td>9</td>
<td>76</td>
<td>14</td>
</tr>
<tr>
<td>&gt;5 yr</td>
<td>14</td>
<td>2</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>

* P = 0.03.

Table 5  Mean values of immunohistochemical staining with confidence intervals (α = 0.05) of all stage IIIA-NSCLC cases (n = 157) and of the cases of cluster 1 (enriched with long survivors; n = 45)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>All cases</th>
<th>Cluster 1</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOS</td>
<td>1.06 (0.90–1.22)</td>
<td>0.45 (0.25–0.64)</td>
<td>−2.4</td>
</tr>
<tr>
<td>N-RAS</td>
<td>1.52 (1.34–1.70)</td>
<td>0.71 (0.43–1.00)</td>
<td>−2.1</td>
</tr>
<tr>
<td>JUN</td>
<td>0.95 (0.80–1.11)</td>
<td>0.51 (0.28–0.74)</td>
<td>−1.9</td>
</tr>
<tr>
<td>Cyclin A</td>
<td>1.68 (1.50–1.87)</td>
<td>1.04 (0.72–1.36)</td>
<td>−1.6</td>
</tr>
<tr>
<td>ERBB1</td>
<td>1.67 (1.51–1.83)</td>
<td>1.18 (0.88–1.48)</td>
<td>−1.4</td>
</tr>
<tr>
<td>PCNA</td>
<td>0.87 (0.67–1.07)</td>
<td>0.63 (0.30–0.96)</td>
<td>−1.4</td>
</tr>
<tr>
<td>FAS/CD95</td>
<td>0.51 (0.42–0.60)</td>
<td>0.44 (0.29–0.60)</td>
<td>−1.2</td>
</tr>
<tr>
<td>PS3</td>
<td>0.94 (0.78–1.10)</td>
<td>0.84 (0.55–1.13)</td>
<td>−1.1</td>
</tr>
<tr>
<td>HIF-1β</td>
<td>2.18 (1.99–2.37)</td>
<td>2.00 (1.60–2.40)</td>
<td>−1.1</td>
</tr>
</tbody>
</table>

enriched in cluster 1 (Table 4). Again, the mean values with confidence intervals of cluster 1 were compared with the data of all stage IIIA carcinomas. FOS, N-RAS, JUN, ERBB1, and cyclin A were significantly down-regulated in the carcinomas of cluster 1 compared with the data of all tumors (Table 5).

Finally, the separation of clusters was carried out with squamous cell lung carcinomas (Table 6). The long-term survivors were enriched in clusters 2 and 4. The expression levels of FOS, JUN, ERBB1, and cyclin A were reduced in carcinomas of patients who survived 5 years in comparison with the expression levels of all patients (Table 7).

In all three analyses (Tables 3, 5, and 7), the expressions of FOS, JUN, ERBB1, and cyclin A or PCNA were decreased in carcinomas of patients who survived 5 years compared with all patients. This suggests the profile of the long-term survivors consisted of factors mainly associated with proliferation.

The relationships between survival and the clusters identified by hierarchical cluster analysis were further analyzed by Kaplan-Meier survival analysis. The survival of all 216 NSCLC patients analyzed is shown in Fig. 2a. As illustrated in Fig. 2b, patients of cluster 3 lived significantly longer than patients of all other clusters (P = 0.006, log-rank; P = 0.027, rank-sum). Comparable data were observed if only stage IIIA tumors were investigated. The overall survival of stage IIIA NSCLC patients is shown in Fig. 2c. As depicted in Fig. 2d, patients of cluster 1 had a significantly better survival probability than patients of all other clusters (P = 0.002, log-rank; P = 0.032, rank-sum).

**DISCUSSION**

Advances in molecular and cellular biology have opened new avenues for the characterization of tumors. Although the Tumor-Node-Metastasis staging system is still the best prognostic factor for NSCLC, the measurement of gene expression may become useful adjuncts to predict outcome for individual patients. The systematic investigation of different cellular factors in NSCLCs may help to identify a specific profile for long-term surviving patients. In clinical practice, the portion of long-term surviving patients suffering from NSCLCs is small, and much efforts have been devoted to new treatment protocols to improve...
survival times (11, 12). On the other hand, the molecular biology of tumors from long-term survivors is not understood at present, and the knowledge of factors that influence long-term survival may be helpful to design new treatment options.

We analyzed the relationships of 21 parameters including proto-oncogene and tumor suppressor gene products and proliferative, apoptotic, and angiogenic factors with the survival times of patients. Of these 21 factors, 9 were statistically significant related to long-term survival of patients (>5 years). There is a great wealth of information on prognostic factors for survival of NSCLC, but the analysis of one or few factors may not suffice the need to understand the complex regulatory cellular networks affecting long-term survival. Therefore, we further analyzed these 9 parameters by hierarchical cluster analysis.

Hierarchical cluster analysis is an explorative statistical method classifying objects by calculation of distances according to the closeness of between-individual distances. The merging of objects with similar features leads to the formation of a cluster, where the length of the branch indicates the degree of relationship. Thus, objects with tightly related features appear together, whereas the separation in the cluster tree increases with progressive dissimilarity. The general applicability of this method has been shown for gene expression profiling and for a method of approaching the molecular pharmacology of cancer (13, 14).

We believe that the analysis of a large number of parameters by immunohistochemistry in combination with hierarchical cluster analysis represents an appropriate approach to generate hypotheses based on expression profiles of genes to determine the biological behavior of tumors. The molecular factors found in the present investigation and their involvement in signaling cascades favor the view that complex pathways drive tumor growth and, thereby, influence long-term survival of NSCLC patients. There are some studies addressing the role of clinical parameters for long-term survival of patients afflicted with NSCLC. A low number of metastatic sites, stage, female gender, and improvements in treatment protocols were found as favorable prognostic factors for long-term survival (11, 15, 16).

The existence of an expression profile of cellular and molecular factors indicative for long-term survivors with NSCLC, as shown in the present study, fuels the hope that it may become realistic to devise therapeutic strategies, making prognoses even better.

Among the parameters identified by hierarchical cluster analyses, the transcription factors FOS and JUN, the epidermal growth factor ERBB1, and the proliferation marker cyclin A or PCNA were significantly related to long-term survival. This suggests that the profiles of long-term survivors consisted of factors mainly associated with proliferation, because all of these parameters are in some way involved in the regulation of proliferation. This indicates that tumor growth is an important determinant that affects long-term survival of patients with lung cancer. An immunohistochemical staining panel consisting of proliferative markers and the cluster analysis-aided profiling of tumors as shown in the present study may, therefore, allow the molecular recognition of long-term surviving patients.

There are now sophisticated bioinformatic tools in hand for the mining of huge data sets; it is ironic that fewer, rather than thousands, of sets of genes may provide more robust results (17). Our study demonstrated the feasibility of hierarchical cluster analysis to identify long-term survivors afflicted with NSCLC using few cellular factors. Although the sequencing of
the human genome provided the basis to search for genes with clinical significance, the determination of genes with prognostic value in individual patients will be the wave of the future in clinical cancer research. The understanding of individual differences in gene expression and the ability to predict survival of patients will have substantial impact on the development of individualized therapy options.

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