Correlation between survivin expression and prognosis in non-small cell lung cancer

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Non-small cell lung cancer; Prognosis; Survivin

Summary
Aims and Background: Survivin is a recently identified protein as an inhibitor of apoptosis, which supresses programmed cell death and regulates cell division. In this study, we investigated the prognostic significance of both nuclear and cytoplasmic survivin expression in non-small cell lung cancer (NSCLC) and examined the association with clinicopathological parameters.

Methods: The study comprised 58 male patients diagnosed NSCLC with a mean age of 57.29±8.82 years; range 40–76 years. Patients underwent lobectomy (64%) or pneumonectomy (36%) with hilar and mediastinal lymph node sampling. Paraffin embedded tumor sections were retrieved for evaluation of nuclear and cytoplasmic staining of survivin. Clinicopathological data, stage and survival of patients were all determined.

Results: Cytoplasmic staining was found significantly increased in squamous cell carcinoma (P=0.003), whereas there was no association between nuclear staining and histopathological type (P=0.837). Also, both nuclear and cytoplasmic staining did not show any association with tumor stage (P>0.05). In univariate analysis there was significant correlation between nuclear survivin and short survival (P=0.0002). In multivariate survival analysis using Cox regression, only nuclear staining of survivin was determined as an independent prognostic factor (P=0.004).

Conclusions: Localization of survivin expression might have an important regulatory mechanism in carcinogenesis and tumor progression. Nuclear survivin expression in
tumor tissues might predict the prognosis in NSCLC, whereas cytoplasmic survivin has no prognostic significance.

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Introduction

Lung cancer is the leading cause of cancer death all over the world and there is no reliable and effective diagnostic molecular markers predicting prognosis in non-small cell lung cancer (NSCLC). Therefore, new molecular markers will allow the development of the novel therapeutic strategies for lung cancer. Survivin is a recently identified protein as an inhibitor of apoptosis, which suppresses programmed cell death and regulates cell division. The expression of survivin is undetectable or found at very low levels in normal tissues, whereas at high levels in various malignancies and also embryonic and fetal tissues. Survivin is expressed in the G2/M phase of the cell cycle and at the beginning of mitosis, survivin associates with microtubules of the mitotic spindle. Hence, the overexpression of survivin may overcome this apoptotic checkpoint and promote aberrant progression of the transformed cell through mitosis.

High level of survivin expression in malignancies is considered as an important indicator of poor prognosis and associated with high tumor grade, tumor progression and chemoresistance. Lung cancer and breast cancer cells express the highest levels of survivin and also survivin expression is correlated with shorter survival in patients with NSCLC. Survivin has both nuclear and cytoplasmic staining in cancer cells which involve in regulation of mitosis and apoptosis. In this study, we aimed to determine the prognostic significance of both nuclear and cytoplasmic expression of survivin in NSCLC and examined the association with clinicopathological parameters.

Materials and methods

Patients

Archived tissue blocks from 2000–2003 were retrieved from the files of the Department of Pathology in Atatürk Chest Diseases and Chest Surgery Education and Training Hospital. The study comprised 58 male patients diagnosed NSCLC with a mean age of 57.29 ± 8.82 years; range 40–76 years. Patients underwent lobectomy (64%) or pneumonectomy (36%) with hilar and mediastinal lymph node sampling. None of the patients received chemotherapy or radiation therapy before surgery. Histopathological diagnosis was carried out according to the WHO/IASLC (1999) classification of lung and pleural tumors. In our study, 36 patients were diagnosed squamous cell carcinoma and 22 patients were adenocarcinoma. Clinical stage was determined according to the TNM staging system (1997). There were 5 patients with stage 1A, 20 patients with stage 1B, 15 patients with stage 2B, 18 patients with stage 3A. The characteristics of patients are shown in Table 1.

Immunohistochemistry

Paraffin embedded material was available in a set of 58 individual tumors for evaluation of nuclear and cytoplasmic staining of survivin. The study was carried out using a standard avidin–biotin–peroxidase complex technique and UltraVision LP
Detection System, with a mouse monoclonal antibody against survivin Ab-2 (Neo Markers, Clone4F7). The 6-μm-thick tissue sections were deparaffinized in xylene 2 h, washed twice in ethanol. Survivin Ab-2 required boiling in 10 mM Citrate buffer pH 6.0 for 20 min at microwave oven. Sections were incubated with the primary antibody solution for survivin Ab-2 at a dilution of 1:50 for 30 min at room temperature. Immunoreaction was completed with the avidin–biotin–peroxidase complex kit (LabVision). After incubation with a diaminobenside (DAB) specimens were counterstained with Harris haematoxylin and cover slipped.

Survivin cytoplasmic immunoreactivity was evaluated semiquantitatively based on the intensity of staining. The percentage of positive tumor cells were evaluated in at least five areas at x 400 and scored as negative; no survivin cytoplasmic staining, 1+ (weak); 0–25% staining, 2+ (moderate); 25–50% staining and 3+ (intense); more than 50% cytoplasmic staining. Positive survivin nuclear immunoreactivity was accepted as more than 25% positive nuclei staining.

Statistical analysis

Statistical analysis was performed using the SPSS for windows release 10.0 package program. Chi-square test was used for comparison of data between the groups. Univariate analysis was performed and the significance of differences in survival between the groups was determined using log rank test. Survival curves were computed according to the method of Kaplan Meier. To evaluate the independent prognostic relevance of nuclear and cytoplasmic staining of survivin multivariate analysis using Cox Regression model was performed. A value of P<0.05 was accepted as statistically significant.

Results

Nuclear and cytoplasmic staining of survivin in tumor tissues

Cytoplasmic survivin staining was weak in 17 (29.3%) specimens, moderate in 21 (36.2%) and intense in 18 (31%) specimens. Also, in 2 (3.5%) specimens cytoplasmic staining of survivin was negative. Nuclear staining of survivin was positive in 28 (48.3) specimens, negative in 30 (51.7%) specimens (Figs. 1–3). There was no correlation between nuclear and cytoplasmic expression of survivin (P = 0.984).

Association with histopathological type and tumor stage

Cytoplasmic staining was found significantly increased in squamous cell carcinoma (P = 0.003), whereas there was no association between nuclear staining and histopathological type (P = 0.837). Also, both nuclear and cytoplasmic staining did not show any association with tumor stage (P > 0.05).
Association between survivin staining and survival

The median survival of patients was determined as $45.1 \pm 6.13$ months. At the end of the study 33 (56.9%) patients had died and 25 (43.1%) patients were alive. There was significant correlation between nuclear staining of survivin and poor survival ($P = 0.0002$), as shown in Table 2 and Fig. 4. Median survival was $49.2 \pm 2.1$ months (95% confidence interval (CI); 45–53.3 months) in patients having negative nuclear survivin staining, whereas $26.7 \pm 8.3$ months (95% CI; 10.3–43 months) in patients with positive nuclear staining. However, there was no significant association between cytoplasmic staining and survival ($P = 0.4034$). Multivariate survival analysis was performed using Cox Regression model. Other prognostic factors as age, tumor diameter, surgery type, histopathology, tumor stage and treatment were all determined. Only nuclear staining of survivin was determined as an independent prognostic factor ($P = 0.004$) (Table 3).

Discussion

Apoptosis is the process in which the damaged cells are eliminated. Tumorigenesis involves a loss of balance between regulators of cells proliferation

Figure 2 Strong cytoplasmic immunoreactivity in an adenocarcinoma.

Figure 3 Strong cytoplasmic and nuclear immunoreactivity in basal cells of squamous cell carcinoma.
and apoptosis. Activation of apoptosis has been shown to be important in lung tumorigenesis and contribute to tumor invasion and metastasis. The newly identified apoptosis inhibitor, survivin, has been shown to be expressed in various human cancers including NSCLC. In this study, we aimed to investigate the clinicopathological and prognostic significance of survivin expression in NSCLC. We demonstrated both nuclear and cytoplasmic staining of survivin in tumor tissues by immunohistochemistry. Survivin could be detected in the majority of resected NSCLC not only as the protein but also as mRNA. Falleni et al. demonstrated survivin mRNA levels higher in squamous cell carcinomas for the first time. Also, we found cytoplasmic survivin significantly increased in squamous cell carcinoma by immunohistochemistry, for the first time. However, there was no association between nuclear survivin and histopathology.

Survivin has dual function in apoptosis and mitosis depending upon its cellular localization and in a number of cancers prognostic significance of nuclear and cytoplasmic survivin expression has been investigated. In our study we found nuclear staining of survivin as a poor prognostic factor in NSCLC similar to the study of Lu et al. However, Vischioni et al. found nuclear localization of survivin as a positive prognostic factor in advanced NSCLC. This finding can be explained by the different stages of the patients, such as in our study we investigated NSCLC patients with early stage, whereas Vischioni et al. analyzed patients with advanced stage. In addition, the second reason might be the methodological differences, such as the technique used to probe survivin.

### Table 2 Prognostic significance of nuclear and cytoplasmic survivin in a univariate analysis using log rank test.

<table>
<thead>
<tr>
<th>Variables</th>
<th>1 year</th>
<th>2 year</th>
<th>3 year</th>
<th>Median ± se</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear (-)</td>
<td>86.6</td>
<td>83.3</td>
<td>79.1</td>
<td>49.2 ± 2.14</td>
<td>0.0002*</td>
</tr>
<tr>
<td>Nuclear (+)</td>
<td>64.2</td>
<td>53.5</td>
<td>35.7</td>
<td>26.7 ± 8.3</td>
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</tr>
<tr>
<td>Cytoplasmic (+)</td>
<td>68.4</td>
<td>57.9</td>
<td>47.4</td>
<td>36.6 ± 18.1</td>
<td>0.403</td>
</tr>
<tr>
<td>Cytoplasmic (++)</td>
<td>76.2</td>
<td>71.4</td>
<td>56.6</td>
<td>49 ± 3.9</td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic (+++)</td>
<td>83.3</td>
<td>77.8</td>
<td>65.8</td>
<td>40.2 ± 4.5</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significant.

### Table 3 Multivariate analysis related to survival using Cox regression.

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.97–1.07</td>
<td>0.387</td>
<td></td>
</tr>
<tr>
<td>Histopathology</td>
<td>0.46</td>
<td>0.18–1.18</td>
<td>0.108</td>
</tr>
<tr>
<td>Tumor diameter</td>
<td>0.95</td>
<td>0.81–1.12</td>
<td>0.593</td>
</tr>
<tr>
<td>Surgery type</td>
<td>1.87</td>
<td>0.67–5.19</td>
<td>0.228</td>
</tr>
<tr>
<td>Stage I (R)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Stage II</td>
<td>1.24</td>
<td>0.41–3.68</td>
<td>0.696</td>
</tr>
<tr>
<td>Stage III</td>
<td>1.85</td>
<td>0.65–5.25</td>
<td>0.247</td>
</tr>
<tr>
<td>Supportive therapy (R)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>0.55</td>
<td>0.12–2.54</td>
<td>0.446</td>
</tr>
<tr>
<td>Chemotherapy*</td>
<td>2.3</td>
<td>0.58–9.14</td>
<td>0.234</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>1.93</td>
<td>0.67–5.58</td>
<td>0.22</td>
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<td>Nuclear survivin</td>
<td>3.73</td>
<td>1.53–9.05</td>
<td>0.004*</td>
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<td>Cytoplasmic (+) (R)</td>
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<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cytoplasmic (++)</td>
<td>0.55</td>
<td>0.18–1.66</td>
<td>0.297</td>
</tr>
<tr>
<td>Cytoplasmic (+++)</td>
<td>0.31</td>
<td>0.1–0.98</td>
<td>0.055</td>
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</table>

*Statistically significant, R: reference category.
expression. Also, Shinohara et al. determined nuclear staining of survivin as a poor prognostic factor in patients with stages I and II resected NSCLC.

The influence of nuclear and cytoplasmic survivin on prognosis vary not only within same tumor types, but also within different tumor types and stages. Kennedy et al. found nuclear survivin as a good prognostic factor in breast cancer, whereas Grabowski et al. showed nuclear survivin associated with poor survival in esophageal cancers. In NSCLC, strong expression of survivin in nucleus might represent increased mitotic events. In contrast, cytoplasmic staining of survivin had no prognostic relevance at all.

Survivin expression might promote both tumor progression and resistance to chemotherapy and irradiation. The molecular mechanisms by which survivin inhibits apoptosis are still under investigation. It has been shown that survivin splice variants had different subcellular localizations and functions. Survivin delta EX3 is localized in nucleus, whereas survivin 2B isoforms are found in cytoplasm. Although survivin delta Ex 3 preserves its antiapoptotic potential, survivin 2B has lost its antiapoptotic feature and also survivin 2B might act as an antagonist of survivin. Ling et al. found high expression level of survivin 2B associated with no relapse and good survival, whereas high expression level of survivin delta Ex 3 was determined as associated with relapse and poor survival.

Survivin is an important predictive factor for recurrence after curative resection in early stage NSCLC. New strategies may be considered with reference to survivin expression to improve treatment of NSCLC after surgical resection. Furthermore, targeted chemotherapy against survivin using monoclonal antibodies might be an approach to treat surviving-positive tumors.

In conclusion, localization of survivin expression might have an important regulatory mechanism for its role in carcinogenesis and tumor progression. Nuclear survivin expression in tumor tissues might predict the prognosis in NSCLC. Further studies comprising more patients should be performed to investigate the prognostic significance of nuclear and cytoplasmic survivin expression in NSCLC.

References


