Abnormal expression and clinicopathologic significance of p120-catenin in lung cancer

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Summary. The aim of this study was to investigate the relationship between the expression of p120ctn in human lung squamous cell carcinoma, adenocarcinoma and its clinicopathologic significance. The expression of p120ctn in tumors and adjacent normal lung tissues from 143 patients was examined by immunohistochemistry and Western blot. Expression of p120ctn occurs mainly in the cell membrane of normal bronchial mucosa. Abnormal expression of p120ctn, including cytoplasmic and reduced membranous expression, was found in 114 of 143 specimens (79.7%) and was significantly associated with poor differentiation, high TNM stage, and lymph node metastasis (P<0.05 for each) but not with histologic subtype. The Kaplan-Meier survival test revealed that abnormal expression of p120ctn was related to poor survival (P<0.001). A Cox regression analysis revealed that abnormal p120ctn expression was an independent factor in predicting patient survival (P=0.024). Compared with that in normal lung tissues, membranous protein level was lower in tumors (P=0.003). Abnormal expression of p120ctn is associated with tumor progression and poor prognosis in lung squamous cell carcinoma and adenocarcinoma. Reduced expression or even the absence of p120ctn isoform 1 and 3 in tumor cell membranes may be responsible for the abnormal expression of p120ctn that has been found in lung cancer.

Key words: Catenin, Lung cancer, p120ctn, Prognosis

Introduction

Lung cancer is one of the most lethal malignant tumors, and the primary causes of this lethality include tumor recurrence and metastasis. Dysfunction of adhesion molecule and destruction of cell adhesion are the initial steps of tumor metastasis. p120-catenin (p120ctn), a newly found adhesion molecule, may play an important role in tumor progression and metastasis. However, there is no data on its isoform expression and significance in lung squamous cell carcinoma (SCC) and adenocarcinoma in Chinese patients.

Originally, p120 ctn was identified as a tyrosine kinase substrate of Src and tyrosine, phosphorylated by several growth factors, such as EGF, PDEF, CSF-1, and Erb-B2 (Anastasiadis and Reynolds, 2000). As a member of the catenin family, which includes ß- and γ-catenin, p120ctn can bind with the cytoplasmic and reduced membranous expression, was found in 114 of 143 specimens (79.7%) and was significantly associated with poor differentiation, high TNM stage, and lymph node metastasis (P<0.05 for each) but not with histologic subtype. The Kaplan-Meier survival test revealed that abnormal expression of p120ctn was related to poor survival (P<0.001). A Cox regression analysis revealed that abnormal p120ctn expression was an independent factor in predicting patient survival (P=0.024). Compared with that in normal lung tissues, membranous protein level was lower in tumors (P=0.003). Abnormal expression of p120ctn is associated with tumor progression and poor prognosis in lung squamous cell carcinoma and adenocarcinoma. Reduced expression or even the absence of p120ctn isoform 1 and 3 in tumor cell membranes may be responsible for the abnormal expression of p120ctn that has been found in lung cancer.

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Several p120ctn isoforms from cell membranous proteins were stored at -70°C immediately after resection (10 samples, 32 fresh specimens and corresponding normal lung tissues were collected between November 2001 and 2002). In the present study, our data demonstrate that p120ctn is an independent prognostic factor for the survival of patients with lung SCC and adenocarcinoma. In a previous paper, we reported that E-cad but not β-catenin was significantly related to shorter survival (Qiu et al., 2002). They concluded that reduced expression of E-cad and β-, α-, and γ-catenin, but not of p120ctn, was associated with tumor progression and significantly shorter survival. To further address this question, we examined the expression patterns of E-cad, β-catenin, and p120ctn and their prognostic significance for 143 patients with lung SCC and adenocarcinoma. In a previous study, Watanabe et al. (2003) identified in 88 of the 143 patients. Among these, lymph node metastases were significantly related to shorter survival (Qiu et al., 2002). In the present study, our data demonstrate that p120ctn is an independent prognostic factor for the survival of patients with lung SCC and adenocarcinoma. Several p120ctn isoforms from cell membranous proteins are for the first time identified in these tumor samples.

Materials and methods

Case materials

This study was conducted with the approval of the local institutional review board at China Medical University. The primary tumors of 143 patients with lung SCC and adenocarcinoma who underwent complete resection (105 males and 38 females) in the First Affiliated Hospital of China Medical University were studied retrospectively. Of these samples, 80 were obtained between 1980 and 2001, and the complete follow-up for each patient was included in the prognosis analysis (ranging from 8 to 140 months); the other 63 samples were collected between November 2001 and September 2002. None of the patients had received radiotherapy or chemotherapy before surgical resection, and all were treated with routine chemotherapy after the operation. The mean age of the patients was 59 years (range, 26-87 years). All 143 specimens were reevaluated with respect to histologic subtype, differentiation, and tumor stage (Travis et al., 2004), which included 79 SCC (18 well differentiated, 28 moderately differentiated, and 33 poorly differentiated) and 64 adenocarcinomas (14 well differentiated, 30 moderately differentiated, and 20 poorly differentiated). The TNM staging system of the International Union Against Cancer (1999) was used to classify the specimens as stage I (n=29), II (n=9), III (n=96), or IV (n=9) (Watanabe, 2003). Lymph node metastases were identified in 88 of the 143 patients. Among these samples, 32 fresh specimens and corresponding normal tissue samples were used for Western blot analysis and were stored at -70°C immediately after resection (10 SCC and 22 adenocarcinomas from 24 males and 8 females; mean age of patients, 59 years).

Immunohistochemical assessment

Surgically excised tumor specimens were fixed with 10% neutral formalin and embedded in paraffin, and 4-μm-thick sections were prepared. Normal bronchial epithelium present in the tumor slides was used as an internal positive control. Immunostaining was performed by the avidin-biotin–peroxidase complex method (Ultrasensitive™, MaiXin, Fuzhou, China). The sections were deparaffinized in xylene, rehydrated with graded alcohol, and then boiled in 0.01 M citrate buffer (pH 6.0) for 2 min with an autoclave. Next, 0.3% hydrogen peroxide was applied to block the endogenous peroxidase activity and the sections were incubated with normal goat serum, to reduce nonspecific binding. They were then incubated with p120ctn-specific mouse monoclonal antibody (1:200 dilution; Transduction Laboratories, Lexington, KY, USA) or mouse immunoglobulin (at the same concentration), as the negative control, at 4°C overnight. Biotinylated goat anti-mouse serum IgG was used as a secondary antibody. After washing, the sections were incubated with streptavidin–biotin conjugated with horse-radish peroxidase, and the peroxidase reaction was developed with 3,3’-diaminobenzidine tetrahydrochloride. Counterstaining was done lightly with hematoxylin, and the sections were dehydrated in alcohol before mounting.

Two investigators who were unaware of the clinical data examined all tumor slides randomly. Five views were examined per slide, and 100 cells were observed per view, at x400 magnification. Labeling scores were determined by the percentage of positive cells per slide (0%–100%), for membranous and cytoplasmic staining separately. In normal bronchial epithelium, p120ctn showed a strong staining pattern in the cell membrane and little staining in the cytoplasm. Tumor tissues showed both staining patterns. Therefore, we semiquantitatively classified all the stained slides into 3 categories, according to the proportion of tumor cells that were immunoreactive for p120ctn. When ≥ 90% of the tumor cells stained for p120ctn in the cell membrane, the case was defined as normal membranous expression. When < 90% of the tumor cells stained for membranous expression, the case was defined as reduced membranous expression. When ≥ 10% of the tumor cells stained for p120ctn in cytoplasm, the case was defined as ectopic cytoplasmic expression. Either reduced membranous expression or ectopic cytoplasmic expression was defined as abnormal expression of p120ctn.

Western blot

Fresh frozen samples from 32 tumors and corresponding normal lung tissues were lysed with lysis buffer (50 mM Tris pH 7.2, 150 mM NaCl, 1 mM EDTA, and 1 mM DTT with 10 µg/ml leupeptin, 10 µg/ml aprotinin, and 1 mM PMSF) and were centrifuged at 12000 g for 20 min at 4°C. The sediment was placed in lysis buffer plus 1% Triton X-100, at 4°C overnight, and then was centrifuged again at 12000 g for 20 min at 4°C to extract membranous protein. Finally, the supernatants were stored at -20°C, and protein...
concentrations were determined by the Bradford method. Forty micrograms of each sample was separated by 12% SDS-PAGE and then was transferred to a polyvinylidene fluoride membrane. The transferred samples were incubated with the anti-p120ctn antibody at a 1:800 dilution, at 4°C overnight. After incubation with peroxidase-coupled anti-mouse IgG (HuaMei, Beijing, China), the proteins were visualized by an enhanced chemiluminescence system (HuaMei, Beijing, China). Autoradiograms were quantified by densitometry, and relative p120ctn protein levels were calculated by comparison with the amount of β-actin protein.

Statistical analysis

SPSS version 11.5 for Windows was used for all analyses. The χ² test was used to examine possible correlations between p120ctn expression and clinicopathologic factors. The Student t test was used to compare data from the densitometry analysis of the Western blots. The Kaplan-Meier method was used to estimate the probability of patient survival, and differences in survival between patient subgroups were further compared by means of the log-rank test. By use of a Cox proportional-hazards regression model, the possible prognostic significances of clinicopathologic variables were tested. Differences were considered significant when P<0.05.

Results

Abnormal p120ctn expression

Expression of p120ctn occurs mainly in the cell membrane of normal bronchial epithelium. Of 143 lung cancers, 29 (20.3%) showed normal or preserved membranous expression only (Fig. 1a), and 114 (79.7%) showed abnormal expression, including reduced membranous expression (Fig. 1b), preserved membranous expression accompanied with ectopic cytoplasmic expression (Fig. 2a), and reduced or absent membranous expression together with ectopic cytoplasmic expression (Fig. 1c, 2b, and 2c).

Of 39 specimens with normal membranous expression, 10 cases (25.6%) showed cytoplasmic expression, while 68 (65.4%) of the 104 specimens with reduced membranous expression showed ectopic cytoplasmic expression (χ²=18.07; P<0.001). No statistical difference was found in reduced membranous expression of p120ctn (χ²=0.86; P=0.354) between adenocarcinomas (76.6%, 49/64) and SCC (69.6%, 55/79). Squamous cell carcinomas (63.3%, 50/79) showed significantly higher percentages of ectopic cytoplasmic expressions compared with adenocarcinomas (43.8%, 28/64, χ²=5.45, P=0.020). In additional experiments, we found that the staining patterns for p120ctn were heterogeneous between bronchiolo-alveolar carcinomas (BAC)-like components and other histological components. Unlike other histological components, the cancer cells in BAC-like region mostly showed normal membrane expression of p120ctn, while only few cells had cytoplasmic and/or
reduced membrane expression of p120ctn.

Next, we examined associations between abnormal expression of p120ctn and clinicopathologic factors (Table 1). We found that abnormal p120ctn expression occurred more frequently in samples with poor differentiation, advanced tumor stage, and lymph node metastasis (P=0.041, P<0.001, and P<0.001, respectively), while no significant associations were found with regard to age, sex and histologic type.

Abnormal p120ctn expression and patient prognosis

The expression of p120ctn and its relation to survival time were further analyzed among 80 patients with complete follow-up data. The patients with normal p120ctn expression had a longer mean survival time (45.73±12.10 months) and a higher 5-year overall survival rate (58.3%). For patients with abnormal p120ctn expression, mean survival time was 24.57±5.06 months and the 5-year overall survival rate was only 5.9%, which were statistically different from the patients with normal p120ctn expression (P<0.001). Using the Kaplan-Meier method, we determined survival curves of lung SCC, adenocarcinoma, and both together (Fig. 3) and then compared them by using the log-rank test, which showed that the patients with abnormal p120ctn expression had a poorer prognosis than those with normal or preserved p120ctn expression (P<0.001 for lung SCC, adenocarcinoma and together). As shown by the results from the Cox proportional-hazards regression model (Table 2), abnormal p120ctn expression, TNM stage, lymph node metastasis, and differentiation were significant predictive factors of overall survival (P=0.024, P=0.002, P=0.048, and P=0.018, respectively). Subgroup analyses with the Wald χ² test also demonstrated that abnormal p120ctn expression, high TNM stage, poor differentiation, and lymph node metastasis indicated a worse chance of survival for patients with lung cancer (P=0.023, P=0.001, P=0.017, and P=0.049, respectively; Table 3). Other factors, such as age and sex, had no significant association with prognosis for patients with lung SCC and adenocarcinoma in this study.

Reduced membranous p120ctn expression and aberrant isoforms

In order to examine p120ctn isoforms and compare membranous p120ctn expression between normal lung tissues and lung cancers, fresh frozen samples from 32 lung cancer specimens and corresponding normal lung tissues were subjected to Western blot. The average level of p120ctn expression in normal lung tissue was significantly higher than that in the lung cancer specimens (3.11±1.74 and 1.77±1.31 respectively; P=0.001, n=32). P120ctn has 4 isoforms (isoforms 1-4). In normal lung tissues, several specific bands that were 120 kDa (corresponding to isoform 1) and 100 kDa (corresponding to isoform 3) appeared, while the lung cancer samples showed faintly stained bands or the absence of isoform 1 and isoform 3 (Fig. 4). Image analysis of Western blot results showed that the levels of

![Fig. 2. Immunohistochemistry of p120ctn in lung squamous cell carcinoma. (a) Normal membranous expression together with weak cytoplasmic staining in a moderately differentiated squamous cell carcinoma. Abnormal expression of p120ctn could be either reduced membranous immunostaining associated with cytoplasmic expression (b) or only cytoplasmic staining without membranous expression (c). x 400](image-url)
isoform 1 and 3 in lung cancer were significantly lower than corresponding normal lung tissues (0.59±0.87 vs 1.40±1.45 for isoform 1, P= 0.005, n=32; 1.40±1.10 vs 1.91±1.04 for isoform 3, P=0.037, n=32).

Additionally, we examined the relationship between the result of immunohistochemistry and western blot analysis using Spearman’s correlation analysis. The results showed that the expression quantities of p120ctn immunohistochemistry were significantly correlated with the total densities of p120ctn bands of western blot analysis (P=0.003, Correlation Coefficient: 0.502; n=32). The reduced membrane expression of p120ctn staining also significantly corresponded to the reduction of p120ctn isoform 1 seen in western blot analysis (P=0.021, Correlation Coefficient: 0.407, n=32). No correlation was seen between membrane expression and isoform 3 (P=0.200); cytoplasmic expression and isoform 1 (P=0.257); or cytoplasmic expression and isoform 3 (P=0.892). Therefore, the p120ctn isoform 1 may be the most important isoform that is relevant to reduced membrane expression of p120ctn in lung cancers.

Fig. 3. Kaplan-Meier curve of the overall survival of 80 patients with lung SCC (a), adenocarcinoma (b) and both (c), stratified by normal or abnormal expression of p120ctn. Abnormal p120ctn expression was significantly correlated with poor survival (all P<0.001).

Table 1. Expression of p120ctn and clinicopathologic factors.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>p120ctn abnormal express in SCC</th>
<th>p120ctn abnormal express in Adenocarcinoma</th>
<th>p120ctn abnormal express in all tumor samples</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60</td>
<td>25 / 35 (71.4%)</td>
<td>21 / 26 (80.8%)</td>
<td>46 / 61 (75.4%)</td>
</tr>
<tr>
<td>≥ 60</td>
<td>30 / 40 (80.0%)</td>
<td>36 / 42 (85.7%)</td>
<td>68 / 82 (82.9%)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>46 / 58 (79.3%)</td>
<td>39 / 47 (83.0%)</td>
<td>85 / 105 (81.0%)</td>
</tr>
<tr>
<td>Female</td>
<td>11 / 17 (64.7%)</td>
<td>18 / 21 (85.7%)</td>
<td>29 / 38 (76.3%)</td>
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<td>Histologic type</td>
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<td></td>
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</tr>
<tr>
<td>SCC</td>
<td></td>
<td></td>
<td>64 / 79 (81.0%)</td>
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<tr>
<td>Adenocarcinoma</td>
<td></td>
<td></td>
<td>50 / 64 (78.1%)</td>
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<tr>
<td>Differentiation</td>
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<td>0.669</td>
</tr>
<tr>
<td>Well or moderate</td>
<td>32 / 45 (71.1%)</td>
<td>35 / 45 (77.8%)</td>
<td>67 / 90 (74.4%)</td>
</tr>
<tr>
<td>Poor</td>
<td>25 / 30 (83.3%)</td>
<td>22 / 23 (95.7%)</td>
<td>47 / 53 (88.7%)</td>
</tr>
<tr>
<td>TNM Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I or II</td>
<td>10 / 21 (47.6%)</td>
<td>9 / 17 (52.9%)</td>
<td>19 / 38 (50.0%)</td>
</tr>
<tr>
<td>III or IV</td>
<td>47 / 54 (87.0%)</td>
<td>48 / 51 (94.1%)</td>
<td>95 / 105 (90.5%)</td>
</tr>
<tr>
<td>Lymphatic metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>17 / 30 (56.7%)</td>
<td>17 / 25 (68.0%)</td>
<td>34 / 55 (61.8%)</td>
</tr>
<tr>
<td>Positive</td>
<td>40 / 45 (88.9%)</td>
<td>40 / 43 (93.0%)</td>
<td>80 / 88 (90.9%)</td>
</tr>
</tbody>
</table>

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Discussion

Altered expression of p120ctn has been implicated in tumor progression in several malignant carcinomas, including cancers of the colon, bladder, stomach, breast, and lung (Thoreson and Reynolds, 2002). In the present study, we found no statistically significant difference in abnormal p120ctn expression between adenocarcinomas and squamous cell carcinomas. Abnormal p120ctn expression was significantly related to poor survival. For the first time, we demonstrate that reduced expression or even the absence of p120ctn isoform 1 and 3 in tumor cell membranes may be responsible for the abnormal expression of p120ctn that has been found in lung cancer. In addition, cytoplasmic expression in SCC was significantly higher than that in adenocarcinomas.

Abnormal expression of p120ctn may damage the function of the E-cad/catenin complex and affects related cell adhesion. As a result, this triggers tumor cells to detach from the primary site, invade surrounding tissues, and metastasize to lymph nodes and distant organs. Acquisition of cell dissociation and motility, induced by aberrations in the E-cad/catenin complex, could thus enhance the release of cancer cells from the primary site and affect the initial steps in the metastasis process. In contrast to one earlier report by Bremnes et al. (2002) our results reveal that abnormal expression of p120ctn is an important pathologic alteration in the tumor metastasis process.

Membranous p120ctn expression in normal tissues may activate Rho GTPase-Cdc42, which can bind to IQGAP1, an effector of Cdc42 (Noren et al., 2000). Free IQGAP1 negatively regulates cell adhesion by dissociating α-catenin from the E-cad/catenin complex and inhibiting the recognition of the cytoskeleton by the complex. Activated Cdc42 may interact with IQGAP1 and suppress its dissociating action. The former can maintain cell adhesion and cell structure. In another unpublished study, we found that, in lung cancers Cdc42 also showed reduced membranous expression or overexpression in the cytoplasm, which has significant consistency with abnormal p120ctn expression (kappa = 0.493, P<0.001, data not shown here).

Thus far, few reports about the nuclear expression of p120ctn in tumors are available. Daniel and Reynolds first observed that, in the cell nucleus, p120ctn could connect with Kaiso, a transcription factor of the POZ/ZF (Pox virus and zinc finger) family (Daniel et al., 1999). Sarrio et al. (2004) only found 4 of 67 cases of nuclear p120ctn expression in breast cancer tumors. We did not find any nuclear p120ctn expression in our samples. Reduced membranous p120ctn expression was also identified in our samples by Western blot analysis. The p120ctn protein levels in cellular membranes were significantly higher in normal lung tissues than in lung cancers. The expression of large amounts of isoforms that have distinct biologic and tissue-specific functions may explain why p120ctn can both positively and negatively affect cell adhesion. We found that isoforms 1 and 3 mainly presented in normal lung tissues, while isoform 1 expression was reduced or absent in lung cancer. The expression levels of p120ctn isoform 1 shown by western blot analysis were positively correlated with p120ctn membrane expression shown by immunohistochemistry. This result was consistent with the report by Montonen et al. and suggested that the expression of p120ctn isoform 1 in cell membranes could help maintain cell structure and adhesion (Montonen et al., 2001).

The abnormal expression of p120ctn has been related to an unfavorable outcome in several human cancers, although conflicting results have been reported. The
abnormal expression of p120\textsuperscript{ctn} has been correlated with poor survival in a study of 68 patients with bladder cancer. On the contrary other studies did not find an association between abnormal p120\textsuperscript{ctn} expression and survival among 61 patients with gastric adenocarcinoma and 193 patients with NSCLC (Brien et al., 1998; Bremnes et al., 2002). In the present study, abnormal expression of p120\textsuperscript{ctn} was associated with poor patient survival. Additionally, Cox regression analysis showed that combination of the immunohistochemical results of p120\textsuperscript{ctn} and E-cadherin was also a significant predictive factor of overall survival in lung cancers (P=0.023). On the basis of our results and those of others, we suggest that abnormal p120\textsuperscript{ctn} expression is related to poor prognosis in tumor clinical stage and lymph node metastasis and p120\textsuperscript{ctn} expression is a significant factor in the prediction of survival for patients with lung cancer.

In summary, we conclude that lung cancers show a reduced membranous expression and ectopic cytoplasmic accumulation of p120\textsuperscript{ctn}. The faintly expressed or absent isoform may be responsible for the reduced membranous p120\textsuperscript{ctn} expression observed microscopically in this study and frequently reported by others. Abnormal expression of p120\textsuperscript{ctn} is associated with poor patient survival and may be useful in predicting the progression of lung cancer. Further experiments are under way to clarify whether ectopic p120\textsuperscript{ctn} expression reflects alterations in the tyrosine kinase pathway.

References


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