The Role of p16-CyclinD1-CDK4/6-Rb Pathway in the Pathogenesis of Non-Small-Cell Lung Carcinoma

Jianming Liu¹, Shenghua Sun¹, Qun Jiang²,*

¹ Department of Respiratory Diseases, The Third Hospital of Central South University, Changsha, Hunan, 410013, China.
² The Second Hospital of Central South University, Changsha, Hunan, 410013, China.

Abstract

Background: The p16-cyclinD1-pRB pathway plays a very important role in the cell cycle regulation. It has been shown that the p16-cyclinD-pRB pathway is closely related to tumor genesis. With the development of study, the research in NSCLC have aroused wide interest and attention.

Objective: To analyze the expression of p16, pRB and cyclin D1 in non-small-cell lung carcinoma (NSCLC), the relationship among the three and the connection of the three kinds of protein with NSCLC clinical and pathological indicators.

Methods: NSCLC specimens were collected from 84 cases seen in our hospital, expression of NSCLC-related genes p16, cyclin D1 and Rb in NSCLC tissues was studied with immunohistochemical and histopathological techniques.

Results: 1. The p16 protein was negative in 32 (38.1%) of the 84 NSCLC cases, weakly positive in 7 cases (8.3%), and medium to strongly positive in 45 cases (53.6%); pRB protein was negative in 43 cases (51.2%), weakly positive in 11 cases (13.1%), and medium to strongly positive in 30 cases (35.7%); over-expression of cyclin D1 was seen in 46 cases (54.8%), weakly positive in 4 cases (4.7%), and negative in 34 cases (40.5%). 2. Expressions of p16 and pRB had a negative correlation; the cyclin D1 over-expression rate in positive groups was significantly higher than that in the pRB negative group. 3. Expression of pRB had a negative correlation with the differentiation degree of NSCLC; expression of p16 was related to the degree of differentiation of adenocarcinoma, good differentiation>moderate differentiation>poor differentiation (P<0.01). Cyclin D1 did not show remarkable correlation with the differentiation degree of NSCLC.

Conclusion: Anomaly of p16-CyclinD1-CDK4/6-Rb pathway may be closely related to the pathogenesis of NSCLC.

Keywords: Non-Small-Cell Lung Carcinoma, p16, cyclin D1, pRB, Immunohistochemistry, Clinical Pathology.

Received: July 26, 2014 Accepted: August 30, 2014 Published: September 30, 2014

Grant support: This work was supported, in part, by grants from the Project (2012TT2029) (2014TT2024) of Hunan Provincial Science and Technology Department; Project (B2012-034) of Hunan Provincial Department of Public Health

*Correspondent: Jiang Qun, The Second Hospital of Central South University, Changsha, Hunan, China. 410013. E-mail: jiangqun126@126.com
**Introduction**

The pRB in the p16-cyclin D-pRB pathway is at low phosphorylated status at the G1 stage, combines with and suppresses transcription factor E2FS, and then prevents the transcription of related genes of S stage, thus causes the cell to stagnate at the G1 stage. cyclin D1 protein forms a compound with CDK4/6 at the G1 stage, inactivates pRB phosphorylation, releases out E2FS, starts up the transcription of related genes at S stage and causes cell division or transformation. At this moment, the p16 level rises and binds CDK4/6 in competition with cyclin D1 and interdicts its phosphorylation on pRB. Therefore, p16 and pRB are negative regulatory factors of the cell cycle, also called as anti-oncogene protein; cyclin D1 and CDK4/6 are positive regulatory factors, also called as oncoprotein. Researchers found that pRB could suppress the expression of its protein through the negative regulation of the INK4A promoter of p16 gene, and in those cells which are absent of functional pRB, the expression level of p16 escalates. And yet p53 pathway induces the expression of p21wafl through activation of p53 gene when the cells are in oxygen deficit and the DNA is damaged, while the latter can suppress various cyclin-CDK compounds and stop evolution of the cell cycle [1, 2]. For different kinds of carcinoma, its changes in molecular biology are different, and the two pathways have different significance in pathogenesis of different carcinomas.

It has been reported in literature that the abnormality rate of p53 in NSCLC is about 15%-35% [3-5]. Abnormality of p16-cyclin D-pRB pathway has been studied for various carcinomas while researches on the performance of p16-cyclin D-pRB pathway in NSCLC can only be seen in the reports of Jin et al [6, 7]. The expressions of p16, pRB and cyclin D1 in NSCLC would be detected by using immunohistochemical method, analysis on the relationship between the three and discussion on the connection of the three kinds of protein with NSCLC clinical pathological indicators will be conducted.

**Materials and Methods**

**Materials**

Eighty-four cases of NSCLC and 20 cases of non-lung carcinoma tissues were selected for this research. The samples were from the paraffin blocks archived from 2003 to 2013 in the Department of Pathology of the Third Xiangya Hospital of Central South University. Types of pathological tissues are classified as per WHO International Histology Taxonomy, and there were 52 cases of squamous carcinoma and 32 cases of adenocarcinoma. The average age of the 84 NSCLC cases (male: female = 62:22) are 55.3 (32-68). Small cell carcinoma and other types of lung carcinoma were not selected because of their particularity and too less case load. Follow-up visits were recorded at the patients’ dates of operation and the finish time was the time of death or the latest return visit of the patients. Post-operative survival time was from 2.5 months to more than 5 years. Materials of non-lung carcinoma tissues of the 20 cases were taken from those patients suffering pulmonary bulla and pneumorrhagia.

**Reagents**

See Table 1 for the summary of the first antibody. SP kits were the products of Zymed, DAB were purchased from Sigma, and the rest reagents are domestic Analytical Pure products.

**Methods**

S-P Method (streptavidin-biotin immunoperoxidase method) was used for immunohistochemical staining to detect related genes p16, cyclin D1 and Rb of cell cycle in the
carcinoma tissues of non-small-cell lung carcinoma and analysis was done in combination with clinical pathological files.

**Comparison**

Given positive sections provided by the kits are taken as positive contrast for the staining of p16, pRB and cyclin D1: in the staining processes of these three kinds of antibodies, PBS is used instead of the first antibody as blank control.

**Interpretation of the results**

Referring to the domestic and overseas literature, appearance of brown immune deposits at cell nucleus was defined as positive, and cytoplasm positive [8-10] should be ignored. Double-blind method was adopted and 10 high power fields (×400) were calculated. Results were semi-quantitatively judged according to the two indicators as the percentage of number of positive cells against that of the counted cells and the tint strength of positive cells in the sections. The percentages were determined according to the number of positive cells against that of the total counted cells and were divided into four grades: 0 point for basically not tinted; 1 point for mild yellow; 2 points for yellowish brown; and 3 points for brownish sepia. Multiply the score of color staining degree with that of cell-percentage for each of the sections and then get its final score. 0~1 point are negative (-); 2~3 points are weakly positive (+); 4~6 points are medium positive (++); and above six points are strongly positive (+++). For the expression of p16 and pRB, being negative is abnormal; and for the cyclin D1 protein, over-expression (++~+++ ) is abnormal.

**Statistical analysis**

SPSS 17.0 was applied for statistical analysis. X inspection was used for analysis on difference or relationship of classified information and t inspection for independent samples was adopted for SPF means. Pearson correlation analysis method or Spearman Rank correlation method was used for analyzing the correlations.

**Results**

**Expression of p16, pRB and cyclin D1 proteins**

The immunoreaction products of p16, pRB and cyclin D1 mainly existed in cell nuclei, parts of which are accompanied with cytoplasm
tinting. Referring to domestic and overseas literature, only nucleus positives were taken into calculation while cytoplasm positives were ignored. Weakly positive expressions presented by p16 and pRB and negative or weakly positive expressions presented by cyclin D1 could be seen in normal lung tissues. p16 protein in 32 out of 84 cases (38.1%) of NSCLC was negative, 7 out of 84 (8.3%) weakly positive, and 45 out of 84 (53.6%) medium to strongly positive; pRB protein in 43 out of 84 cases (51.2%) was negative, in 11 out of 84 (13.1%) weakly positive (+) and in 30 out of 84 (35.7%) medium to strongly positive (++~+++); 46 out of 84 cases (54.8%) had cyclin D1 over-expressed (++++), 4 out of 84 cases (4.7%) weakly positive (+) and 34 out of 84 (40.5%) negative.

See Figure 1 to 7 for the three kinds of protein immunohistochemical staining. Rate of being negative for p16 and pRB had significant difference compared to the control groups and the NSCLC groups (X2 inspection, P<0.05) (Table 2).

**Correlation of p16, pRB and cyclin D1**

In the 43 cases of NSCLC, in which pRB was negative, 41 cases had positive expression of p16, and 2 cases were weakly positive; and in the 32 cases of NSCLC, p16 was negative, 26 cases were pRB positive and 6 cases weakly positive; besides, 9 cases were pRB and p16 proteins both weakly positive or positive (Table 3, Figure 8), p16 showed a negative correlation with the expression of pRB (data processing was done by utilizing Pearson correlation analysis and Spearman rank correlation analysis and all correlation coefficients were -0.706 (P<0.01).

See Table 4 for the mutual relations of expression of cyclin D1 protein with p16 and pRB. In the 41 cases of NSCLC, in which pRB was positive (+++++), 31 cases had over-expression of cyclin D1 and 10 cases normal expression, thus the rate of over-expression was 75.6%; and in the 43 cases of NSCLC, in whom pRB was negative, 15 cases over-expressed cyclin D1, 28 cases normally expressed, thus the rate of over-expression was 34.9%. After statistical analysis, the over-expression rate of cyclin D1 of the pRB positive group was apparently higher than that of pRB negative group (X2=6.016, P<0.05).

On the other hand, in the 52 cases of NSCLC, in whom p16 was normal (+++++), 25 cases had over-expression of cyclin D1, 27 cases normally expressed, thus the rate of over-expression was 48.1%; and in the 32 cases of NSCLC, in whom p16 was negative, 23 cases had over-expression of cyclin D1, 9 cases normally expressed, thus the rate of over-expression was 71.2%. Although the cyclin D1 over-expression rate of the p16 negative group was higher than that of the p16 positive group, after having been processed with statistics, the difference of the two groups showed no significant differences (X2=1.575, P>0.05).

See Figure 9 and Figure 10 for schematic diagram of the relationship between cyclin D1 expression and pRB & p16.

**Relationship between expressions of p16, pRB&cyclin D1 and NSCLC clinical pathology**

The pRB absence rate (66.7%) of poorly-differentiated NSCLC is apparently higher than that of well-differentiated NSCLC (30.8%) and the difference therein has remarkable significance (Table 5), which indicates that pRB expression showed negative correlation with the differentiation degree of NSCLC; the poorer the carcinoma cells differentiate, the higher the pRB absence rate is. The expression of p16 relates to the differentiation degree of adenocarcinoma, while good differentiation > moderate
Figure 1. In normal lung tissues, p16 staining of bronchial mucous epithelium, alveolar epithelium, alveolar septum blood vessel endothelium and fibroblast appear positive. (200x).

Figure 2. p16 protein of lung squamous carcinoma cell is distributed in nucleus (200x).

Figure 3. p16 protein of lung adenocarcinoma cell appears nucleoplasm distribution and p16 protein of mesenchyma cell appears positive (200x).

Figure 4. Expression of pRB in lung squamous carcinoma (200x).

Figure 5. Expression of cyclin D1 in adenocarcinoma cell(200x).

Figure 6. Positive expression of p16 protein in lung squamous carcinoma(200x).
Figure 7. Negative expression of p16 in adenocarcinoma (200×).

Table 2. Immunohistochemical results of p16, pRB and cyclin D1 protein

<table>
<thead>
<tr>
<th>Group</th>
<th>p16</th>
<th>pRB</th>
<th>cyclin D1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>A (%)</td>
<td>N</td>
</tr>
<tr>
<td>Normal (20)</td>
<td>10</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>NSCLC (84)</td>
<td>52</td>
<td>32 (38.1)</td>
<td>41</td>
</tr>
</tbody>
</table>

Note: N-Normal, A-Abnormal. Appearing negative of p16 and pRB is abnormal, appearing over-expressed of cyclin D1 is abnormal.

Table 3. The relationship between p16 and pRB

<table>
<thead>
<tr>
<th>p16</th>
<th>pRB (-)</th>
<th>pRB (+)</th>
<th>pRB (++~+++)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p16 (-)</td>
<td>0</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>p16 (+)</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>p16 (++~+++)</td>
<td>41</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 4: The relationship between the expression of cyclin D1 and pRB and p16

<table>
<thead>
<tr>
<th>cyclin D1</th>
<th>pRB (N)</th>
<th>pRB (A)</th>
<th>Subtotal</th>
<th>pRB (N)</th>
<th>pRB (A)</th>
<th>Subtotal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Expression</td>
<td>p16(N)</td>
<td>p16(A)</td>
<td>subtotal</td>
<td>p16(N)</td>
<td>p16(A)</td>
<td>subtotal</td>
<td></td>
</tr>
<tr>
<td>Overexpression</td>
<td>9</td>
<td>10</td>
<td>25</td>
<td>0</td>
<td>28</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Overexpression</td>
<td>23</td>
<td>31</td>
<td>18</td>
<td>0</td>
<td>15</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>32</td>
<td>41</td>
<td>43</td>
<td>43</td>
<td>84</td>
<td></td>
</tr>
</tbody>
</table>

Note: N-Normal, A-Abnormal. Appearing negative of p16 and pRB is abnormal, +~+++ is normal.

Figure 8. The relationship between p16 and pRB (P<0.05)

Figure 9. Relationship between cyclin D1 and pRB expression in NSCLC
Table 5. Correlation between p16, pRB, Cyclin D1 and clinical pathology of NSCLC

<table>
<thead>
<tr>
<th>Item</th>
<th>n</th>
<th>p16</th>
<th></th>
<th>pRB</th>
<th></th>
<th>Cyclin D1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival time recorded in&lt;24months</td>
<td>58</td>
<td>46 12</td>
<td>24 34</td>
<td>8 50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follow-up visits</td>
<td>26</td>
<td>7 (20.1)</td>
<td>11 15</td>
<td>9 17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td>Male</td>
<td></td>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>62</td>
<td>31 19</td>
<td>26 36</td>
<td>26 36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>15 7 12</td>
<td>8 7</td>
<td>3 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;=50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>69</td>
<td>34 8</td>
<td>28 41</td>
<td>30 39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNM</td>
<td></td>
<td>I-II</td>
<td></td>
<td>III-IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>7 35</td>
<td>7 8</td>
<td>3 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well differentiation</td>
<td>71</td>
<td>35 8</td>
<td>28 43</td>
<td>31 40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell</td>
<td></td>
<td>Moderate differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>13</td>
<td>6 5</td>
<td>7 6(46.2)</td>
<td>6 7(53.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor differentiation</td>
<td>18</td>
<td>10 (38.5)</td>
<td>6 12(66.7)*</td>
<td>9 9(50.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well differentiation</td>
<td>11</td>
<td>2 7(53.8)</td>
<td>7 4(36.4)</td>
<td>4 7(63.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td></td>
<td>Moderate differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor differentiation</td>
<td>14</td>
<td>12 8(44.4)</td>
<td>8 7(46.7)</td>
<td>7 8(53.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3(20)</td>
<td>0(0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X2=4.627, P<0.05
N-Normal, A-Abnormal. Appearing negative of P16 and pRB is abnormal; Appearing over-expressed of Cyclin D1 is abnormal
differentiation > poor differentiation and \( P<0.01 \), which means that it has no relation with the differentiation of squamous cell carcinoma. Cyclin D1 appeared to have no significant correlation with the differentiation degree of NSCLC, but the p16 absence rate in the well-differentiated group of squamous cell carcinoma and the rate of cyclin D1 overexpression in the groups of squamous cell carcinoma and adenocarcinoma were quite high, which indicates that abnormal expression of the two may possibly occur at the early stage of NSCLC.

Discussion

The role of p16-cyclin D-pRB pathway in cell cycle control

p16 is a 15.8 KD protein genetically coded by INK4a (also known as CDKN2 & MTS1) on chromosome 9p21 and contains 156 amino acids [11]. Cyclin D1 contains 295 amino acids and is coded by gene CCND1 located on llq13. pRB is a 105 KD protein [13,14] coded by retinoblastoma gene (Rb). These three kinds of protein participate in the regulation of G1/S stage transition of cell cycle. Its mechanism of control has basically been made clear through the studies by many scholars. Figure 11 gives a schematic sign of the role of p16-cyclin D-pRB pathway in the transition of G1/S stage. pRB is at its low phosphorylation status in G1 stage and suppresses a group of transcription factors named as E2Fs in combination with the “pouch area” of protein structure to further stop the transcription of related genes of S stage, thus allows the cell to stay in G1 stage and to fail G1/S stage transition [12]. Cyclin D1 activates the kinase activity of CDK after having formed a compound with CDK4/6, which can promote pRB to generate phosphorylation inactivation and release E2Fs, start transcription of the genes related to S stage, and further cause cell division or conversion [13]. And p16 binds CDK4/6 in competition with cyclin D1 and interdicts its phosphorylation towards pRB [11]. Therefore, both p16 and pRB are negative regulatory factors for the cell cycle and are also called as anti-oncogene protein; both cyclin D1 and CDK4/6 are positive regulatory factors and are also called as oncoprotein. A good deal of evidences indicate that any malfunction in any link of p16-cyclin D-pRB pathway may possibly bring about disorder in the cell cycle control and cause oncogenesis.

Expression of p16, pRB and cyclin D1 proteins in NSCLC

In this group of NSCLC, the total abnormality of p16, cyclin D1 and pRB is 83 out of 84 cases (98.8%), in which 32 cases (38.1%) showed p16 negative, 43 cases (51.2%) appear pRB negative and 46 cases (54.8%) appear cyclin D1 over-expressed. Only one case had normal expression of all the three proteins. The above results indicate that the abnormality of p16-cyclin D-pRB pathway is closely related to the pathogenesis of NSCLC and, in the meantime those results also support such opinion as that oncogenesis is not the result of change of some single gene/protein, but the consequence
of malfunction of the functional pathway consisted of a variety of ingredients.

In NSCLC, the rate of being negative of p16 and pRB (respectively 38.1% and 51.2%) is prominently higher than that of normal pulmonary tissue (0%), which indicates that both can be taken as molecular biological referencing indicator for identification of benign/malignant pulmonary lesions. On the other hand, since the expression of p16 and pRB is still normal in most of NSCLC, overall consideration should be taken into account in combination with conventional light glass observation and biological behavior etc. during discrimination of benign/malignant cancer. Kashiwabara et al [15] had discovered that, absence of p16 protein expression was quite common (66%) in NSCLC, and in this article, the rate (38.1%) of being negative of p16 in NSCLC is close to the result (26.1%) provided by Kashiwabara et al, while the rate (47.6%) of being negative of pRB is lower than the 70% reported by Kashiwabara et al; and the possible reasons for this are such factors as regional difference of carcinoma, difference of sampling quantity and difference of the used antibodies, etc.. Presently, it is believed that the mechanism causing p16 protein to be negative is mainly the absence and mutation of p16 INK4a gene and the aberrant methylation of promoter region. Further research will be conducted in the second part.

The immunohistochemical results of this article indicates that over-expression (54.8%) of cyclin D1, compared to the abnormality of p16 and pRB, is a more common molecular abnormality in NSCLC; even if it may take place in benign tumor (55.0%), it is possible to mainly reflect proliferation status of the cells and is an early event in generation of tumor. The research on squamous cell carcinoma at the head and neck regions done by Kyomoto et al [16] and the studies done by Gansauge et al [17] on pancreatic carcinoma also come to the same conclusion. A good deal of evidences indicates that over-expression of cyclin D1 may allow the cells to easily step over the G1/S checkpoint and thus cause generation of tumor. In parathyroidoma, gene CCND1 of cyclin D1 frequently transpositions to the downstream of the promoter of parathyrin gene due to pericentric inversion, and had over-expression of protein under its control. In mantle cell lymphoma, heavy chain enhancer of immune globulin often transfers to the locus of CCND1 and can also promote protein synthesis and over-expression of cyclin D1. Yet in other kinds of tumor, over-expression of cyclin D1 is mainly caused by the amplification of CCND1 gene. So, in NSCLC, is the amplification of gene CCND1 the main reason for causing over-expression of cyclin D1? Further research will be conducted in the third part.

Relationship between the three kinds of protein

In this group of NSCLC, obvious negative correlation exists between the expressions of p16 and pRB, which is consistent [18-21] with the results detected by other authors in such tumors as esophagus cancer, thymoma and breast cancer, etc. This kind of negative correlation reflects the mechanism of interaction of p16 and pRB in control of cell cycle. pRB may suppress p16 protein expression through its negative regulatory effect against p16 gene promoter, and when functional pRB is absent, this suppression is relieved and protein level of p16 rises. In NSCLC which is reflected as pRB negative in immunohistochemical staining, p16 appears medium to strongly positive expression. Functions of pRB situates at the downstream of p16 in p16-cyclin D-pRB pathway, so when functional pRB is absent, even if the expression level of p16 is rising, the evolution of cell cycle cannot be effectively held back. Negative correlation of p16 and pRB also indicates that molecular abnormality of any of the two will possibly cause out-of-
control proliferation of cells of G1 stage. In the nine cases of NSCLC, in which both p16 and pRB are normal, eight cases appear over-expression of cyclin D1, which suggests that the biological effect of cell proliferation promoted by cyclin D1 may exceed the suppression effect by p16 and pRB to the cell cycle. It can be seen from the above analysis that absences of the three, i.e. proteins p16 and pRB and over-expression of cyclin D1, may alone or jointly participate in the formation of NSCLC.

In the experiment, the over-expression rate (75.6%) of cyclin D1 in the pRB positive group is apparently higher than that (34.9%) of pRB negative group, which indicates that cyclin D1 and pRB expression possess positive correlation and having the back of the opinion [22] proposed by some scholars that low-phosphorylated pRB can stimulate transcription of cyclin D1. In the pRB negative cases of the NSCLC group reported by Kashiwabara et al [15], there have never been any over-expression of cyclin D1, and the author considers that over-expression of cyclin D1 depends on functional pRB. And in our cases of NSCLC, in which pRB is negative, 34.9% are detected as having over-expression of cyclin D1, which shows that in this part of NSCLC, over-expression of cyclin D1 may possibly promote tumor cell growth via other ways not depending on pRB, and both kinds of abnormalities can exist at the same time.

**Connection of p16, cyclin D1 and pRB with clinical pathology**

In this study, it has been discovered that the pRB absence rate (66.7%) of poorly-differentiated NSCLC was significantly higher than that (30.8%) of well-differentiated NSCLC, which indicates that the expression of pRB and the differentiation degree of NSCLC present a negative correlation, suggesting that it may possibly be a molecular event in which development of tumor is at a later stage, participate in the evolution of NSCLC and can be taken as a molecular biological indicator to assist in the discrimination of grade malignancy of NSCLC. p16 and cyclin D1 show no prominent correlation with the differentiation degree of NSCLC, but may still suggest higher abnormality rate of the two in the well-differentiated group, which prompts that abnormality of the two may possibly occur at the early stage of NSCLC. Comparing p16 (-) pRB (+) with p16 (+) pRB (+), there are more (P<0.001) III–IV stages of TNM staging, in which the cell proliferation activity is stronger and the survival time is shorter (P<0.01). Comparing p16 (-) pRB(-) with p16(+)pRB(-), there are more (P<0.001) III–IV stages of TNM staging, but no significant difference of survival time relating to both of them can be seen. This group of information shows that p16 and pRB expressions indicate a negative correlation (P<0.05); while comparing cyclinD1+p16 (-) pRB(+), there are more III–IV stages (P<0.001) of TNM staging, the survival time which is shorter (P<0.01), and at the same time have better proliferation activity. With respect to histopathology, comparing p16 (-) pRB (-) with p16 (+) pRB (-), there are more adenocarcinoma (P<0.05), and viewing cyclin D1 + pRB(+), there are more adenocarcinoma. Both possess had statistical significance.

**References**


18. Jin M, Inoue S, Umemura T, Moriya J, Arakawa M, Nagashima K, Kato H. Cyclin D1, p16 and retinoblastoma gene product expression as a predictor for...