Expression and Significance of CK5/6, P63, P40, CK7, TTF-1, NapsinA, CD56, Syn and CgA in Biopsy Specimen of Squamous Cell Carcinoma, Adenocarcinoma and Small Cell Lung Carcinoma

Expresión y Significado de CK5/6, P63, P40, CK7, TTF-1, NapsinA, CD56 Syn y CgA en Muestras de Biopsia de Carcinoma de Células Escamosas, Adenocarcinoma y Carcinoma de Células Pequeñas de Pulmón

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SUMMARY: Nine tumor and various potential biomarkers were measured and combined the information to diagnose disease, all patients accepted fiber bronchoscopy brush liquid based cytology and histopathology examination in order to reliably detect lung cancer. The samples from 314 Chinese lung cancer patients were obtained and CK5/6, P63, P40, CK7, TTF-1, NapsinA, CD56, Syn and CgA were measured with the immunohistochemical SP method and analyzed correlation of the expression of these markers with pathological and clinical features of squamous cell carcinoma, adenocarcinoma, and small cell lung carcinoma. Squamous cell carcinoma, adenocarcinoma and small cell carcinoma were 61 cases, 114 cases and 139 cases, CK5/6 and P63 expression were more frequent in squamous cell carcinoma, with sensitivity and specificity of 77.05 % and 96.44 %, 83.61 % and 88.93 %, and compared with adenocarcinoma and small cell carcinoma difference was statistically significant (P<0.05). The incidences of a positive P40 expression were 100 % in squamous cell carcinoma, with specificity of 98.81 %.CK5/6 and P63 expression were more frequent in adenocarcinoma, with sensitivity and specificity of 85.09 % and 78.69 %, 79.82 % and 93.44 %, 56.14 % and 95.08 %, and compared with squamous cell carcinoma and small cell carcinoma difference was statistically significant (P<0.05). TTF-1, Syn, CgA and CD56 expression were more frequent in adenocarcinoma, with sensitivity and specificity of 86.33 % and 93.44 %, 89.21 % and 98.36 %, 74.10 % and 100 %, 96.40 % and 96.72 %. The combined detection of CK5/6, P63 and P40 were more useful and specific in differentiating squamous cell carcinoma. CK7, TTF-1 and NapsinA were more useful and specific in differentiating lung adenocarcinoma. The impaired CD56, TTF-1, Syn and CgA reflects the progression of small cell lung cancer.

KEY WORDS: Lung cancer; Histopathology; Diagnostic value; Biomarkers.

INTRODUCTION

Lung cancer remains the most lethal cancer, and it is the leading cause of mortality of all cancers worldwide (Terry et al., 2010; She et al., 2013). The most common type of lung cancer is lung adenocarcinoma. In humans, squamous cell carcinoma is currently the second most frequent histologic subtype of lung cancer and is the leading subtype of lung cancer in China. With the development of molecular biology techniques as well as increased understanding of the mechanism of lung cancer, molecule targeted therapy or combined therapy gradually becomes the mainstream treatment, which requires pathologists to be able to accurately classify different types of lung cancer. Some cases of lung cancers can not be diagnosed by microscopic observation of histology and require additional confirmation by immunohistochemistry. Simulation studies suggest that CK5, p63, and p40 are commonly used immunohistochemical markers for squamous cell carcinoma, and napsin A (NAPA) and thyroid transcription factor 1 (TTF-1) are markers for adenocarcinoma of the lung. Syn and CgA are markers for small cell carcinoma of the lung cancer. Therefore, we performed immunohistochemistry testing on biopsy samples from 314 patients. Through the detection of expression of CK5/6, P63, P40, CK7, TTF-1, NapsinA CD56, Syn and CgA in squamous cell carcinoma, adenocarcinoma of lungs and small cell lung carcinoma. We aim to find a simple set of specific combination of antigens for the accurate diagnosis.

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MATERIAL AND METHOD

Sample collection. Patients with lung cancers were recruited from First Hospital affiliated with Inner Mongolia Medical University during June 1st, 2013 to Sept. 1st, 2015. Samples from 314 patients were collected, including 61 cases of squamous cell carcinoma of lungs, 114 cases of adenocarcinoma of lungs and 139 cases of small cell lung carcinoma. All patients have undergone histopathological examination.

Immunostaining. Biopsies were taken from the 314 patients with lung cancer at sites where pathological changes occurred. After fixation, dehydration, embedding, slicing and Hematoxylin and Eosin (H&E) staining, samples were observed under the microscope. SF methods were used for slice Immunohistochemistry. PBS was used as a negative control against primary antibodies and known positive adenocarcinoma of lungs, squamous cell carcinoma of lungs and small cell lung carcinoma were used as positive controls. Antibodies (DakoCytomation and antibodies dilutions 1:100) for CK5/6, p63, P40, CK7, TTF-1, NapsinA, CD56, Syn and CgA were purchased from Maxim Biotechnology Development Co. Ltd, using PV-9000 kit and DAB developing system. Slides were reviewed and analyzed by at least 2 pathologists. Diagnosis was chiefly based on histopathology results.

Results determination. Slides under going Immunohistochemical treatment demonstrated positive signals by showing yellow or brown particles. CK5/6, CK7, CD56, Syn and CgA are mainly expressed on cell membrane and cytoplasm; P63, P40 and TTF-1 are mainly expressed nucleus and NapsinA are mainly expressed in cytoplasm. Samples are considered negative if no stain is visible; (+) if less than 25 % cells are stained; (++) if 25 % to 75 % cells are stained and (+++) if more than 75 % cells are stained (Tables I and II).

Statistical methods. Data management and statistical analysis were performed using SPSS13.0 statistical software. One-way ANOVA with Bonferroni post hoc comparison. P<0.05 is considered to be statistically significant.

RESULTS

The sensitivity and specificity of CK5/6, P63 in squamous cell carcinoma of lung cancer is 77.05 % and 96.44 %, 83.61 % and 88.93 % respectively, which has statistical significance compared to adenocarcinoma of lungs and small cell lung carcinoma (P<0.01). The positive rate of P40 in squamous cell carcinoma of lungs is 100 % and its specificity is 98.81 %. The sensitivity and specificity of CK7, TTF-1, NapsinA in adenocarcinoma of lung cancer are 85.09 % and 78.69 %, 79.82 % and 93.44 %, 56.14 % and 95.08 % respectively. The positive expression rate of CK7 and TTF-1 in squamous cell carcinoma of lungs, adenocarcinoma of lungs and small cell lung cancer is statistically significantly different (p<0.01). The sensitivity and specificity of TTF-1, Syn, CgA and CD56 in small cell lung cancer are 86.33 % and 93.44 %, 89.21 % and 98.36 %, 74.10 % and 100 %, 96.40 % and 96.72 % respectively (Figs. 1, 2 and 3).

Table I. Immunophenotyping analysis of lung cancer.

| Antibody | Squamous-cell carcinoma | adenocarcinoma | Small cell carcinoma of lung |
|----------|-------------------------|---------------|----------------------------|
|          | + | - | Positive % | + | - | Positive % | + | - | Positive % |
| CK7      | 13 | 24 | 35.14 | 97 | 1 | 98.98 | 8 | 6 | 57.14 |
| TTF-1    | 4 | 51 | 7.27 | 91 | 17 | 84.26 | 120 | 11 | 91.60 |
| P63      | 51 | 4 | 92.73 | 23 | 53 | 30.26 | 5 | 15 | 25.00 |
| CK5/6    | 47 | 3 | 94.00 | 6 | 84 | 6.67 | 16 | 1 | 15.79 |
| CD56     | 2 | 8 | 20.00 | 1 | 6 | 14.29 | 134 | 1 | 99.26 |
| Syn      | 1 | 12 | 7.69 | 1 | 10 | 9.09 | 124 | 13 | 90.51 |
| P40      | 15 | 0 | 100.00 | 2 | 21 | 8.70 | 1 | 5 | 16.67 |
| NapsinA  | 3 | 25 | 10.71 | 64 | 16 | 80.00 | 0 | 8 | 0.00 |
| CgA      | 0 | 9 | 0.00 | 0 | 11 | 0.00 | 103 | 30 | 77.44 |

Table II. The clinicopathologic characteristics and Immunophenotyping analysis of SqCC, ADC, and scc.

| SqCC | ADC | scc |
|------|-----|-----|
| P63  | TTF-1 | Syn  |
| CK5/6| napsinA | CgA  |
| P40  | TTF-1  | CD56 |
| CK7  |        |      |
|      |        |      |

| Sensitivity % | Specificity % |
|---------------|---------------|
| 83.61         | 88.93         |
| 77.05         | 96.44         |
| 82.59         | 96.44         |
Fig. 1. Immunostaining pattern of CK5/6+, P63+ and P40+ in squamous cell carcinomas.

Fig. 2. Immunostaining pattern of CD56+, CgA+ and Syn+ in small cell lung carcinomas.

Fig. 3. Immunostaining pattern of NapsinA+, CK7+ and TTF-1+ in adenocarcinoma.
DISCUSSION

Traditional histological techniques include enzyme histology, immunohistochemistry, immunofluorescence, tissue microarray, in situ hybridization, fluorescence in situ hybridization, laser microanatomy, electron microscopy, etc. We performed immunohistochemistry testing on biopsy samples from 314 patients. Through the detection of expression of CK5/6, P63, P40, CK7, TTF-1, NapsinA, CD56, Syn and CgA in squamous cell carcinoma, adenocarcinoma of lungs and small cell lung carcinoma.

CK5/6 is a type of cell keratin mainly expressed on basal cells and spinous cells in skin, which however is rarely expressed in glandular epithelium. In our research, the sensitivity of diagnosing CK5/6 squamous cell carcinoma, adenocarcinoma of lungs and small cell lung carcinoma are 77.05 %, 5.26 % and 2.16 %, which is consistent with the 75 %-100 % sensitivity reported in literature (Terry et al.; She et al.; Gurd et al., 2015). P63 is selectively expressed in epithelial basal cells with potential for differentiating into squamous epithelium and myoepithelial cells, therefore, it is plausible to use P63 as a marker to diagnose squamous cell carcinoma in lungs. Previous experiments have demonstrated that P63 is negative in small cell lung carcinoma and has a diagnosis sensitivity of 100 % (Mukhopadhyay & Katzenstein, 2011; Ao et al., 2014). Our data show that the sensitivity and specificity of P63 for diagnosis of squamous cell carcinoma of lungs are 83.61 % and 88.93 % but the sensitivity and specificity of p63 for diagnosis of lung adenocarcinoma and the small cell lung carcinoma are low. This information corresponds to the positive rate of P63 in squamous cell carcinoma of lungs adenocarcinoma of lungs (93.8 % and 8.8 % respectively) reported by literature (Brunnström et al., 2013). Therefore it is possible to use CK5/6 and P63 as individual markers for diagnosis of squamous cell carcinoma, adenocarcinoma of lungs and small cell lung carcinoma. In addition, P40 in 315 samples showed a positive rate of 100 % and is more specific in comparison with P63 and CK5/6. P40 had the highest accuracy in terms of diagnosing poorly-differentiated squamous carcinoma of lungs (Bishop et al., 2012). Combining the detection of CK5/6, P63 and P40 will possibly provide new diagnostic methods for differentiating between morphologically ambiguous poorly-differentiated squamous cell carcinoma of lungs and other cancer types.

CK7 is another type of cell keratin expressed in most normal glandular epithelium cells and transitional epithelium cells but it’s not expressed in squamous epithelium (Kim et al., 2013). Our results show that the sensitivity and specificity of CK7 in adenocarcinoma of lungs are 85.09 % and 78.69 %. CK7’s expression is low in squamous cell carcinoma of lungs and small cell lung carcinoma. In addition, the expression of CK7 is significantly different (P<0.01) in squamous cell carcinoma, adenocarcinoma of lungs and small cell lung carcinoma, which can facilitate the diagnosis of the three types of lung cancer. TTF-1 is a specific molecular marker of thyroid follicular epithelium and alveolar epithelium. Our data show that TTF-1 has high sensitivity and specificity in diagnosis of adenocarcinoma of lungs (79.82 % and 93.44 % respectively). Also, TTF-1 expression is significantly different (P<0.01) in adenocarcinoma and squamous cell carcinoma of lungs, which can serve as sole immunological parameter for differentiating between the two pathological types. NapsinA is a member of aspartic protease family with high specificity, whose expression can be found positive in some TTF-1 negative poorly-differentiated lung adenocarcinoma (She et al.). Our data show that NapsinA are positive in 56.14 % adenocarcinoma in lungs with a specificity of 95.08 % yet rarely positive in adenocarcinoma and squamous cell carcinoma of lungs. TTF-1 was positive in 120 out of 139 cases small cell lung carcinoma while NapsinA was negative in all 139 cases of small cell lung carcinoma. Therefore, TTF-1 in conjunction with NapsinA will be contributive to the clinical diagnosis of adenocarcinoma of lungs and small cell lung carcinoma. Meanwhile, TTF-1 and NapsinA are more specific than CK7, while CK7 is more sensitive than TTF-1 and NapsinA in terms of diagnosis of adenocarcinoma of lungs. CK7 and TTF-1 and NapsinA therefore can serve as immunological marker set for diagnosis of adenocarcinoma of lungs.

CD56, a type of cell adhesion molecules (CAM) is a glycoprotein mostly expressed in neurotendr derived cells, tissue and tumor. It is a marker for diagnosis of neuroendocrine tumors. In our research CD56 had the highest sensitivity of 96.4 % in terms of diagnosing small cell lung carcinoma. The expression of CD56 is higher in small cell lung carcinoma than other neuroendocrine markers, therefore, CD56 is a very stable protein marker (Wang et al., 2002). TTF-1 is a sensitive and specific immunologic marker for diagnosing lung cancer. In our experiment, TTF-1’s sensitivity and specificity are 79.82 % and 93.44 %, 86.39 % and 93.44 % in adenocarcinoma of lungs and small cell lung carcinoma respectively, which is in accordance with literature reports (Chhieng et al., 2001). The high sensitivity and specificity of TTF-1 in the adenocarcinoma of lungs and small cell lung carcinoma makes it a key molecular marker for diagnosing adenocarcinoma and small cell lung carcinoma but it cannot differentiate between these two types of lung cancer. Syn and CgA are traditional neuroendocrine immunological marker often used in diagnosis of neuroendocrine tumors (Kontogianni et al., 2005). Our results show that Syn and CgA have sensitivity and specificity of 89.21 % and 98.36
% 74.1 and 100% respectively. The specificity is high but the sensitivity is low, corresponding to relevant research. Therefore, it possesses the potential of being assistive parameter. Summarizing the above, CD56, Syn and CgA all have relatively high specificity in small cell lung carcinoma and can be utilized in conjunction in clinical practice to improve the diagnosis accuracy of small cell lung carcinoma.

This paper examined the expression of immunological markers CK5/6, P63, P40, CK7, NapsinA, TTF-1, CD56, Syn and CgA in lung cancer tissues and discovered the clinical applicability of these antibodies to differentiate different types of lung cancer histopathology.

PALABRAS CLAVE: Cáncer de pulmón; Histopatología; Valor diagnóstico; biomarcadores.

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