

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

Jing-yuan Wang¹; Xue-min Wang²; Xiao-yan Xu¹; Shi-rong Li¹ & Xiu-lan Liu¹

WANG, J. Y. ; WANG, X. M.; XU, X. Y.; LI, S. R. & LIU, X. L. 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. *Int. J. Morphol.*, 38(2):247-251, 2020.

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 %. CK7, TTF-1 and NapsinA 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.

¹ Basic Medical College of Inner Mongolia Medical University, Hohhot, 010059, China.

² Prison of Hohhot, Hohhot 010059, China.

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 site 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 control. 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			Smallcell 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	3	16	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	CK5/6	P40	CK7	TTF-1	napsinA	TTF-1	Syn	CgA	CD56
Sensitivity %	83.61	77.05	24.59	85.09	79.82	56.14	86.33	89.21	74.10	96.40
Specifity %	88.93	96.44	98.81	78.69	93.44	95.08	93.44	98.36	100	96.72

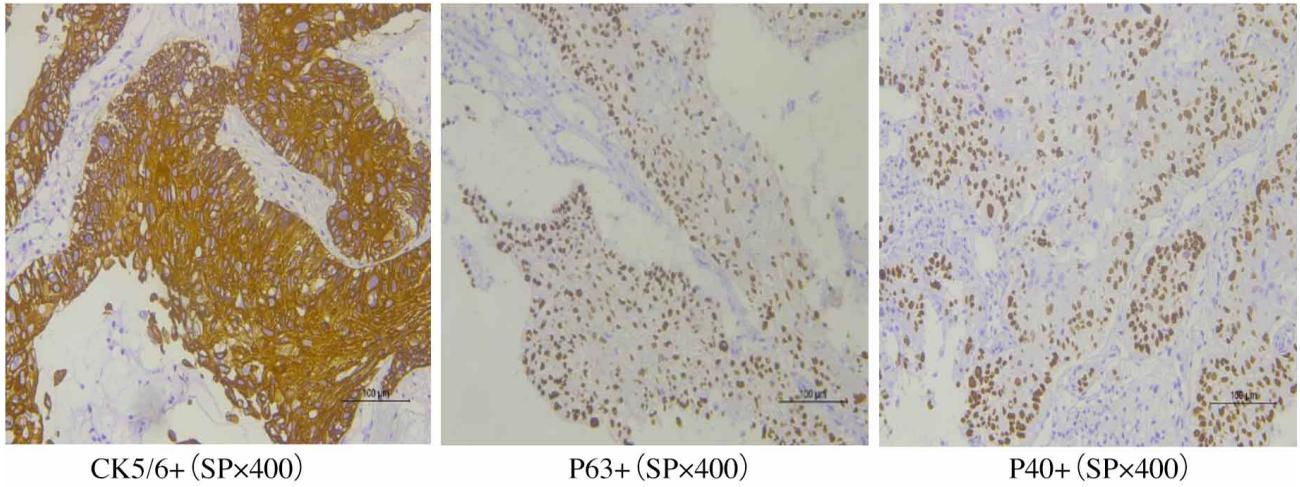


Fig. 1. Immunostaining pattern of CK5/6+, P63+ and P40+ in squamous cell carcinomas.

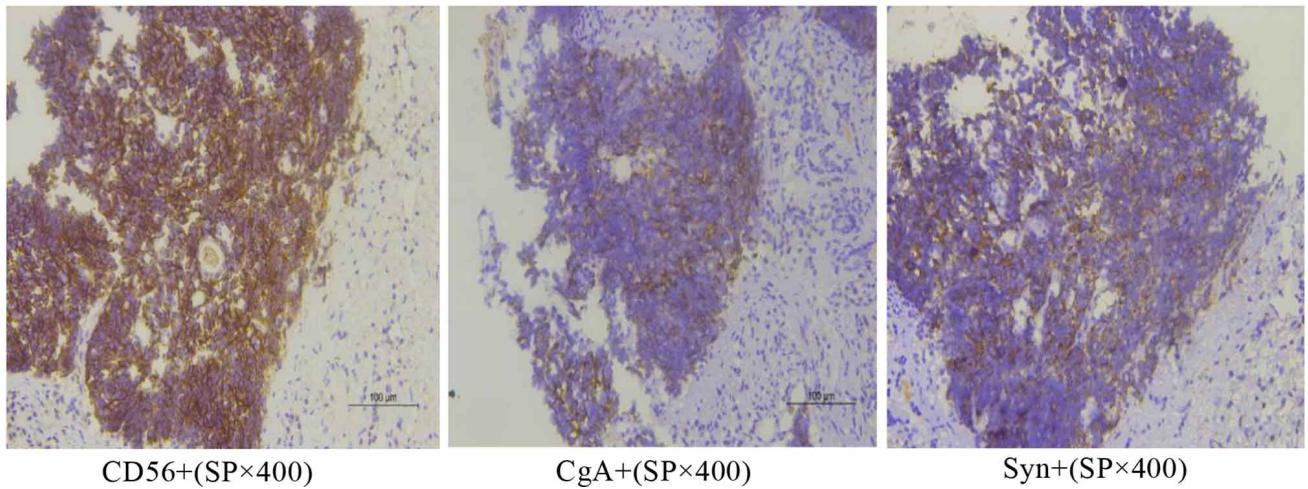


Fig. 2. Immunostaining pattern of CD56+,CgA+ and Syn+ in small cell lung carcinomas.

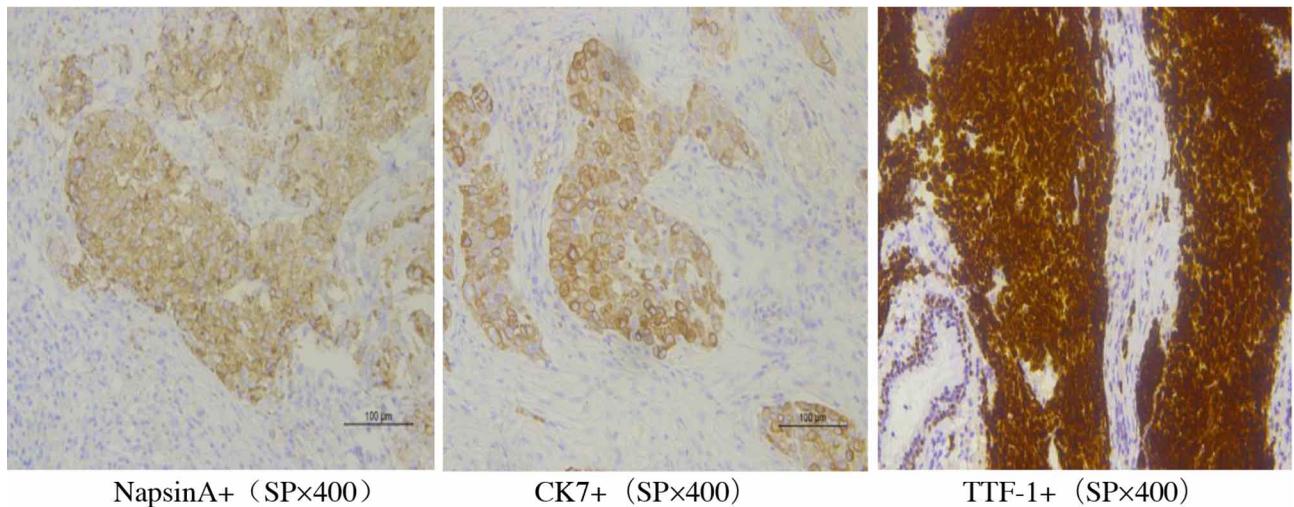


Fig. 3. Immunostaining pattern of NapsinA+,CK7+ and TTF-1+ in adenocarcinoma.

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.*; Gurda *et al.*, 2015). P63 is selectively expressed in epithelium 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 our 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 proteinase 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, 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 neuroderm 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 (Chheng *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.

WANG, J. Y. ; WANG, X. M.; XU, X. Y.; LI, S. R. & LIU, X. L. 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 pulmón de células pequeñas. *Int. J. Morphol.*, 38(2):247-251, 2020.

RESUMEN: Se midieron tumores y utilizaron nueve biomarcadores potenciales y se analizó la información para diagnosticar la enfermedad. A todos los pacientes se les realizó citología en líquido con broncoscopia de fibra y examen histopatológico para detectar de manera confiable el cáncer pulmonar. Se obtuvieron muestras de 314 pacientes chinos con cáncer de pulmón y CK5 / 6, P63, P40, CK7, TTF-1, NapsinA, CD56, Syn y CgA se midieron a través de histoquímica SP y analizaron la correlación de la expresión de estos marcadores con características patológicas y clínicas de carcinoma de células escamosas, adenocarcinoma y carcinoma de células pequeñas en el cáncer de pulmón. El carcinoma de células escamosas, el adenocarcinoma y el carcinoma de células pequeñas fueron 61 casos, 114 casos y 139 casos, respectivamente, la expresión de CK5 / 6 y P63 fueron más frecuentes en el carcinoma de células escamosas, con una sensibilidad y especificidad del 77,05 % y 96,44 %, 83,61 % y 88,93 %, y en comparación con el adenocarcinoma y el carcinoma de células pequeñas, la diferencia fue estadísticamente significativa (P <0,05). La incidencia de ap la expresión positiva P40 fue del 100 % en el carcinoma de células escamosas, con una especificidad del 98,81 %. La expresión de CK7, TTF-1 y NapsinA fueron más frecuentes en el adenocarcinoma, con una sensibilidad y especificidad del 85,09 % y 78,69 %, 79,82 % y 93,44 %, 56,14 % y 95,08 %, y en comparación con el carcinoma de células escamosas y la diferencia de carcinoma de células pequeñas fue estadísticamente significativa (P <0,05) .TTF-1, Syn, CgA y la expresión de CD56 fueron más frecuentes en adenocarcinoma, con sensibilidad y especificidad de 86.33 % y 93.44 %, 89.21 % y 98.36 %, 74.10 % y 100 %, 96.40 % y 96.72 %. La detección combinada de CK5 / 6, P63 y P40 fue más útil y específica en la diferenciación del carcinoma de células escamosas. CK7, TTF-1 y NapsinA fueron más útiles y específicos para diferenciar el adenocarcinoma de pulmón. El deterioro de CD56, TTF-1, Syn y CgA refleja la progresión del cáncer de pulmón de células pequeñas.

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

REFERENCES

- Ao, M. H.; Zhang, H.; Sakowski, L.; Sharma, R.; Illei, P. B.; Gabrielson, E.; Askin, F. & Li, Q. K. The utility of a novel triple marker (Combination of TTF1, Napsin A, and p40) in the subclassification of non-small cell lung cancer. *Hum. Pathol.*, 45(5):926-34, 2014.
- Bishop, J. A.; Teruya-Feldstein, J.; Westra, W. H.; Pelosi, G.; Travis, W. D. & Rektman, N. p40 (DNP63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. *Mod. Pathol.*, 25(3):405-15, 2012.
- Brunnström, H.; Johansson, L.; Jirström, K.; Jönsson, M.; Jönsson, P. & Planck, M. Immunohistochemistry in the differential diagnostics of primary lung cancer: an investigation within the Southern Swedish lung cancer study. *Am. J. Clin. Pathol.*, 140(1):37-46, 2013.
- Chhieng, D. C.; Cangiarella, J. F.; Zakowski, M. F.; Goswami, S.; Cohen, J. M. & Yee, H. T. Use of Thyroid Transcription Factor 1, PE-10, and Cytokeratins 7 and 20 in discriminating between primary lung carcinomas and metastatic lesions in fine-needle aspiration biopsy specimens. *Cancer*, 93(5):330-6, 2001.
- Gerda, G. T.; Zhang, L.; Wang, Y.; Chen, L.; Geddes, S.; Cho, W. C.; Askin, F.; Gabrielson, E. & Li, Q. K. Utility of five commonly used immunohistochemical markers TTF-1, Napsin A, CK7, CK5/6 and P63 in primary and metastatic adenocarcinoma and squamous cell carcinoma of the lung: aretrospective study of 246 fine needle aspiration cases. *Clin. Transl. Med.*, 21(4):16, 2015.
- Kim, M. J.; Shin, H. C.; Shin, K. C. & Ro, J. Y. Best immunohistochemical panel in distinguishing adenocarcinoma from squamous cell carcinoma of lung: tissue microarray assay in resected lung cancer specimens. *Ann. Diagn. Pathol.*, 17(1):85-90, 2013.
- Kontogianni, K.; Nicholson, A. G.; Butcher, D. & Sheppard, M. N. CD56: a useful tool for the diagnosis of small cell lung carcinomas on biopsies with extensive crush artefact. *Clin. Pathol.*, 58(9):978-80, 2005.
- Mukhopadhyay, S. & Katzenstein, A. L. A. Subclassification of non-small cell lung carcinomas lacking morphologic differentiation on biopsy specimens: utility of an immunohistochemical panel containing TTF-1, Napsin A, p63, and CK5/6. *Am. J. Surg. Pathol.*, 35(1):15-25, 2011.
- She, J.; Yang, P.; Hong, Q. & Bai, C. Lung cancer in China: challenges and interventions. *Chest*, 143(4):1117-26, 2013.
- Terry, J.; Leung, S.; Laskin, J.; Leslie, K. O.; Gown, A. M. & Ionescu, D. N. Optimal immunohistochemical markers for distinguishing lung adenocarcinomas from squamous cell carcinomas in small tumor samples. *Am. J. Surg. Pathol.*, 34(12):1805-11, 2010.
- Wang, B. Y.; Gil, J.; Kaufman, D.; Gan, L.; Kohtz, D. S. & Burstein, D. E. P63 in pulmonary epithelium, pulmonary squamous neoplasms, and other pulmonary tumors. *Hum. Pathol.*, 33(9):921-6, 2002.

Corresponding author:

Xiu-lan Liu

Basic medical college of Inner Mongolia Medical University
Hohhot 010059

CHINA

E-mail: wangjingyuanmng@163.com

Received: 25-06-2019

Accepted: 09-10-2019