Histological Subtyping of Lung Carcinomas: From the Targeted Therapy Perspective

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ABSTRACT

Objective: As a result of recent advances in therapies, the subtyping of non-small cell lung carcinomas (NSCLC) has become more important. This study was an evaluation of the use of cytomorphological characteristics and immunohistochemical markers to predict the subtype of NSCLC in fine-needle aspiration (FNA) material.

Methods: Records of 73 cases of surgically resected NSCLC that had been preoperatively diagnosed with FNA biopsy were reviewed. Cytology specimens were reviewed for cytomorphological features of squamous cell carcinoma (SCC) and adenocarcinoma (AC), and the contribution of immunohistochemistry to histological subtyping was evaluated.

Results: The sensitivity, specificity, and positive predictive values for keratinized lamellar cytoplasm and dense chromatin in SCC, and for flat sheets and nucleocytoplasmic polarity in AC were more than 60%. Immunohistochemical analysis revealed 60% sensitivity and 93% specificity for thyroid transcription factor 1 in AC. P63 and cytokeratin 5/6 had 73% and 64% sensitivity and 78% and 96% specificity, respectively, in SCC. The immunohistochemistry results of the cell blocks and the resection material demonstrated 94% conformity.

Conclusion: Immunohistochemistry is helpful in subtyping NSCLC, including poorly differentiated tumors. The cell block method of representing the immune profile of the tumors was found to be reliable.

INTRODUCTION

Lung cancer is one of the most frequently seen and deadliest types of cancer.[1] Since the treatment approach involves chemotherapy in small cell lung cancer (SCLC), and curative radical surgery in non-small cell lung cancer (NSCLC), it is important to classify lung cancers as SCLC or NSCLC.[2,3] Most patients with NSCLC are diagnosed at an advanced stage and lose the chance of surgical treatment. Chemotherapy protocols with adjuvant radiotherapy are performed for these patients; however, the rate of survival is low.[4]

In recent years, in parallel with advances in molecular biology, targets for new treatments have been defined and promising results have been seen in NSCLC. Therapeutic superiority and complications of each agent have been defined according to histological subtype.[5–8] Therefore, knowledge of the histological subtype is of great impor-

tance. Most of the time, cytological material is the only diagnostic tool in this patient group. Therefore, the work of the pathologist has become more difficult and even more valuable.

In this study, cytomorphological characteristics of samples from a patient group diagnosed as NSCLC based on the histological examination of transthoracic fine-needle aspiration biopsy (TFNAB) material who underwent resection were reviewed to evaluate its use in the determination of histological subtype. Immunohistochemical (IHC) study was performed for those with cell blocks, and the contribution of the immune profile to histological typing and the success of the block in representing brachial tissue was analyzed.

MATERIAL AND METHODS

Records of patients who were diagnosed as NSCLC based

on histopathological examination of TFNAB material and who subsequently underwent resection (lobectomy or pneumonectomy) during the period between January 1, 2000 and June 30, 2010 were screened.

Cytological samples were re-evaluated for important cytomorphological characteristics observed in adenocarcinoma (AC), and squamous cell carcinoma (SCC). Characteristics of tumoral cell patterns (single cells, single-layer cover, compact groups, syncytial structures, flow pattern, nucleocytoplasmic polarity, intracellular cell signaling), cell size and shape (small, midsize, large, giant, cylindrical, elongated, polygonal, pleomorphic, single keratinized), nuclei (size; shape; hypo- or hyperchromasia; dense, coarse, or fine granular chromatin; single or multiple nucleoli; eosinophilic nucleoli; intranuclear pseudoinclusion), and cytoplasmic features (narrow, midsize, large, pale, vacuolated, clear, dense, lamellar, keratinized) were analyzed in detail. The presence of mitosis or necrosis was also noted.

The contribution of IHC examination to histological subtyping in cytological samples with a cell block was evaluated. IHC characteristics of cell blocks and resection material were compared, and the representation of tumoral characteristics was reviewed.

Statistical analysis

Cytomorphological characteristics were statistically analyzed using SPSS for Windows, Version 15.0. (SPSS Inc., Chicago, IL, USA). Categorical data were compared using chi-square test. Sensitivity, specificity, and positive predictive value of immunohistochemical antibodies were calculated based on the following formulas:

Sensitivity: True positive / true positive + false negative x 100

Specificity: True negative / true negative + false positive x 100

Positive predictive value: True positive / true positive + false positive x 100

RESULTS

Cytology and resection samples of a total of 73 (male: n=59, 80.8%; female: n=14, 19.2%) patients aged between 29–81 years (mean: 61±10.25 years) were analyzed in the study. The samples of resected material were classified as AC (n=43; 58.9%), SCC (n=22; 30.1%), sarcomatoid carcinoma (n=5; 6.9%: 2 pulmonary blastoma, 2 sarcomatoid carcinoma, 1 pleomorphic carcinoma), large-cell carcinoma (n=2; 2.7%), and adenosquamous carcinoma (n=1; 1.4 %).

In the detailed cytomorphological evaluation of the prepared slides, more than 60% sensitivity, specificity, and positive predictive value was observed for presence of keratinized lamellar cytoplasm, dense hyperchromatic nuclei,
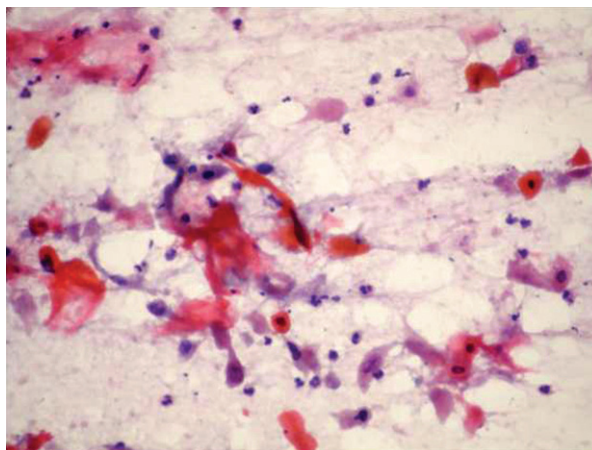

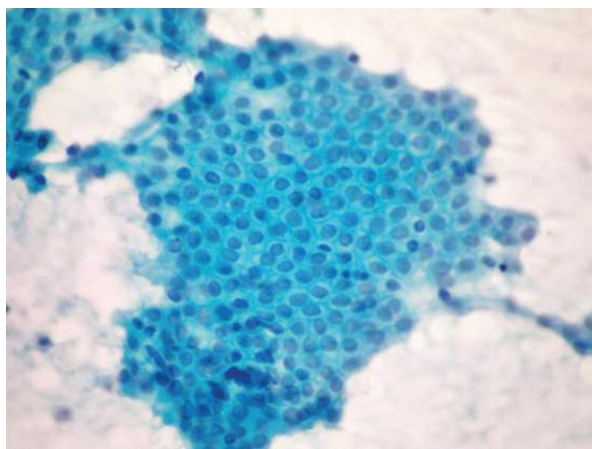**Figure 1.** Well-differentiated keratinized single cells (PAP, x400).

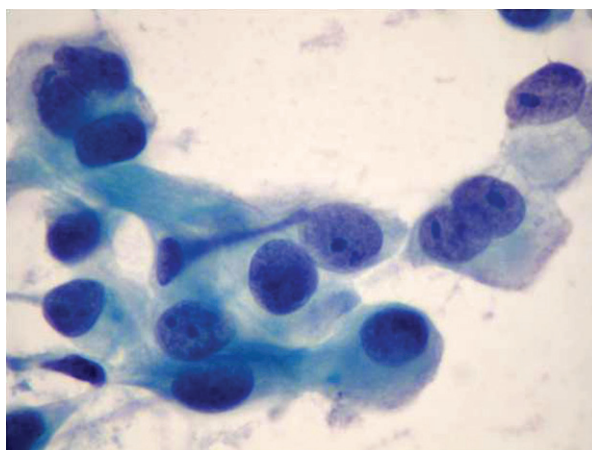**Figure 2.** Well-differentiated adenocarcinoma. Single-layer cover (PAP, x200).

Figure 3. Well-differentiated adenocarcinoma. Nucleocytoplasmic polarity (PAP, x400).

and pleomorphic polygonal cells in SCC, and for single-layer cover and nucleocytoplasmic polarity in AC (Figures 1–3). When the degree of tumor differentiation was taken into consideration, lower degree of sensitivity and

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Table 1. The correlation between cytomorphological criteria and the degree of differentiation in squamous cell carcinoma and adenocarcinoma

Cytomorphological criteria	Squamous cell cancer (n=21)		Adenocarcinoma (n=42)			
	Moderately	Moderately differentiated	Poorly differentiated	Dominancy of lepidic and papillary pattern	Dominancy of acinar pattern	Other
	n=14 (%)	n=7 (%)	n=15 (%)	n=12 (%)	n=6 (%)	n=9 (%)
Prismatic cubic round cell	8 (57)	5 (71)	14 (93)	12 (100)	6 (100)	8 (89)
Foamy vacuolar clear cytoplasm	7 (50)	6 (86)	13 (87)	11 (92)	5 (83)	6 (67)
Elongated spindle-shaped nucleus	11 (79)	6 (86)	12 (80)	8 (67)	3 (50)	8 (89)
Pleomorphic nucleus	5 (36)	5 (71)	11 (73)	4 (33)	3 (50)	4 (44)
Fine granular chromatin	7 (50)	5 (71)	13 (87)	8 (67)	5 (83)	6 (67)
Coarse granular chromatin	13 (93)	7 (100)	13 (87)	6 (50)	5 (83)	5 (56)
Presence of nucleoli	10 (71)	6 (86)	12 (80)	10 (83)	5 (83)	8 (89)
Necrosis	8 (57)	4 (57)	7 (47)	2 (17)	1 (17)	3 (33)
Spindle-shaped elongated cell	10 (71)	6 (86)	6 (40)	2 (17)	2 (33)	5 (56)
Opaque dense chromatin	9 (64)	4 (57)	2 (13)	1 (8)	1 (17)	3 (33)
Pleomorphic polygonal cell	7 (50)	6 (86)	1 (7)	3 (25)	0	3 (33)
Single-layer cover	4 (29)	4 (57)	12 (80)	12 (100)	5 (83)	7 (78)
Compact groups	12 (86)	6 (86)	5 (33)	5 (42)	2 (33)	3 (33)
Nucleocytoplasmic polarity	1 (7)	2 (29)	10 (67)	7 (58)	5 (83)	6 (67)
Glandular structures	0	0	2 (13)	3 (25)	2 (33)	1 (11)
Papillary structures	0	0	5 (33)	6 (50)	3 (50)	2 (22)
Intranuclear pseudoinclusion	1 (7)	0	3 (20)	7 (58)	1 (17)	1 (11)
Keratinized lamellar cytoplasm	11 (79)	5 (71)	0	1 (8)	0	0
Flow	7 (50)	3 (43)	0	0	0	0
Keratinized single cells	8 (57)	4 (57)	0	0	0	0

specificity was noted in poorly differentiated carcinomas; however, these morphological criteria are still important (Table 1).

In all, 37 (50.7%) TFNAB specimens were accurately subtyped, while 6 (8.2%) were inaccurately subtyped, based on cytomorphological characteristics. Histological subtype of the remaining 30 (41.1%) samples could not be determined, and were interpreted as NSCLC.

Thirty-three (45.2%) cytological samples had appropriate cell blocks for IHC evaluation. Sensitivity and specificity for thyroid transcription factor 1 (TTF-1) antibody in AC were determined to be 63% and 93%, respectively. Sensitivity and specificity for p63 was 73% and 64%, and 82% and 96% for cytokeratin (CK) 5/6 antibodies in SCC. Sensitivity and specificity for TTF-1 antibodies in AC resection material was 68% and 93%, respectively, while sensitivity and specificity for p63 was 91% and 73%, and 82% and 96% for CK5/6 antibodies in SCC.

IHC profiles of 31 of 33 (94%) cell blocks were consistent with those of the resected tumor material (Figures 4–6).

In 14 cytological samples with cell blocks and defined as NSCLC, the IHC panel consisting of TTF-1, p63, and CK5/6 aided in the subtyping of 4 of 5 cases of SCC, and 4 of 8 cases of AC (Table 2).
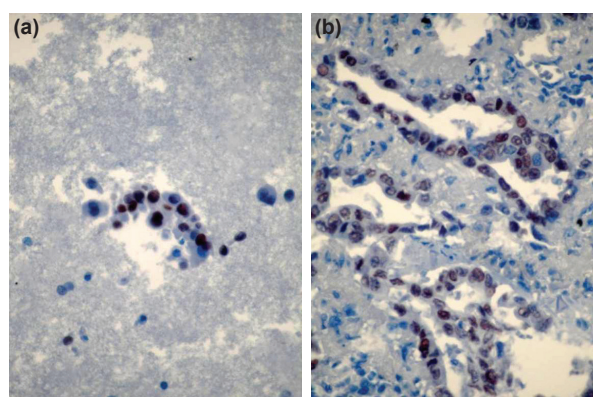

Figure 4. Thyroid transcription factor 1-positive adenocarcinoma (**[a]** Cell block, x400; **[b]** Resection material, x400).
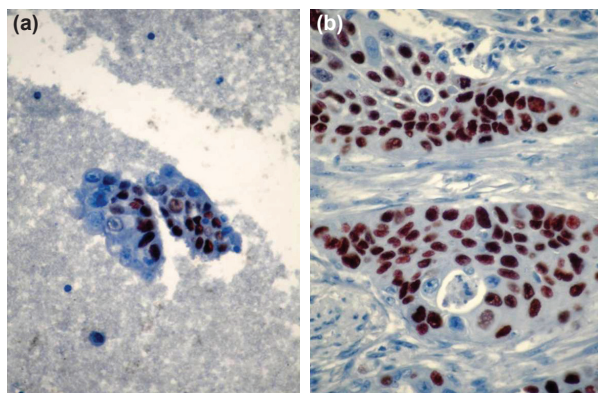
Figure 5. P63-positive squamous cell carcinoma (**[a]** Cell block, x400; **[b]** Resection material, x400).
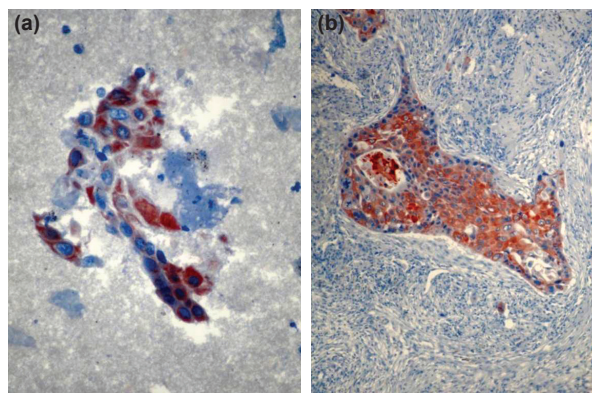

Figure 6. Cytokeratin 5/6 and squamous cell carcinoma (**[a]** Cell block, x400; **[b]** Resection material, x200).

Table 2. Immunohistochemical profile of poorly differentiated tumors classified as non-small cell lung carcinoma

Immunohistochemical profile	Histological tumor type			
	Squamous cell carcinoma n=5 (%)	Adenocarcinoma n=8 (%)	Large-cell carcinoma n=1 (%)	Total n=14
TTF-1 (-) p63 or CK5/6 (+)	4 (80)	0	0	4
TTF-1 (+) p63 and CK5/6 (-)	0	4 (50)	0	4

TTF: Thyroid transcription factor; CK: Cytokeratin.

DISCUSSION

In recent years, as a result of advances in the study of cancer biology, targeted treatments have drawn attention, and the histological subtyping of NSCLC has become more important. This is due to the development of new agents for each histological subtype, which have different therapeutic effects. Therefore, the importance of both small biopsy specimens and cytological material has increased, and the histological subtyping of these samples has become compulsory.[1,5–8]

This study focused on the most frequently seen morphological and IHC characteristics that can be helpful to detect SCLC and AC in the patient group with advanced stage NSCLC.

Morphologically, SCC is characterized by the presence of keratin, while AC is characterized by glandular (adenoid) differentiation.[9] In this study, there was greater than 60% sensitivity, specificity, and positive predictive value for the presence of keratinized lamellar cytoplasm and the distribution of opaque, dense chromatin in the diagnosis of SCC and single-layer cover and nucleocytoplasmic polarity in AC.

Morphological characteristics are very valuable in the diagnosis of moderately and well-differentiated SCC and AC.[9,10] SCC is more accurately diagnosed in cytology ma-

terial than AC. Edwards et al.[11] compared preoperative cytological and postoperative histological diagnoses, and reported diagnostic accuracy rate for SCC and AC of 64% and 32%, respectively. In our study, the diagnostic accuracy was 66.7% for SCC and 56.1% for AC.

Though pulmonary cytology is successful in the discrimination between well-differentiated and moderately differentiated tumors, morphological typing of poorly differentiated tumors remains confusing and controversial. As the degree of differentiation decreases, SCC can assume morphological characteristics that mimic those of AC.[9] Therefore, especially for small biopsy specimens and cytological samples without the characteristics of SCLC in which differentiation could not accurately be determined, the term NSCLC (not otherwise classified) has been recommended and accepted.[3] According to the literature, this nomenclature has been used for nearly 47% of cytological samples.[11] In the present study, this term was used for 37% of the cytological samples.

In poorly differentiated carcinoma where morphological typing is not possible, IHC analysis can aid in the determination of histological subtype. Examination for TTF-1 in AC and combined use of p63 and CK5/6 antibodies in SCC is reliable.[12–16] In lung AC, the presence of TTF-1 has been reported in between 19% and 89% of patients. [1,12,13,17] The wide range in incidence rate in the literature

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may be associated with various degrees of differentiation in tumors in the study series or the characteristics of the antibody clones used. Poorly differentiated pulmonary AC expresses less TTF-1 compared with well-differentiated AC.[18] Furthermore, different TTF-1 antibody clones reportedly affect the rate of positivity.[19]

It has also been underlined that the TTF-1 antibody used in the discrimination between AC and SCC has demonstrated positivity in 0% to 33% of SCC cases.[14,15]

In our study group, the sensitivity and specificity of TTF-1 antibody in AC was 63% and 93%, respectively.

The p63 protein, which is one of the diagnostic markers for lung cancer, is expressed in non-neoplastic squamous-metaplastic epithelium in situ, and in invasive squamous neoplasias.[20]

Therefore, it is used to detect squamous differentiation in cases of poorly differentiated carcinoma[12] P63 positivity has been reported in 78% to 100% of cases of pulmonary SCC.[12–16,20,21]

Though it is a marker specific to squamous cell carcinoma, the presence of p63 has also been reported in 0% to 65% of AC cases.[12,15,20,22]

In our series, the sensitivity and specificity of the p63 antibody in SCC was 73% and 82%, respectively.

Use of the CK5/6 antibody recommended to determine squamous differentiation demonstrates an immune profile similar to that of p63 protein.[12] CK5/6 expression has been reported in 47% to 100% of SCC cases.[12,13,15–17,21] However the presence of CK5/6 positivity has been observed in 0% to 56.2% of AC cases.[12,13,15,16,21]

In our study group, the sensitivity and specificity of the CK5/6 antibody was found to be 64% and 96%, respectively.

None of the markers used in the diagnosis of lung carcinoma is specific for a certain tumor type. Therefore, it has been recommended that tumors that do not demonstrate morphological differentiation should be reported as NSCLC, and the histotype predicted by IHC should be included in the interpretation. However, it is also known that some poorly differentiated tumors classified as NSCLC based on light-microscopic examination cannot not be classified despite further IHC analysis.[13]

The markers we used in our study did not yield excellent outcomes in the determination of specific histological subtype in cases of NSCLC; however, a panel consisting of TTF-1, p63, and CK5/6 aided in accurate subtyping of 4 of 5 (80%) SCC cases and 4 of 8 (50%) AC cases morphologically reported as NSCLC in patients who had no chance of surgery and were scheduled for chemotherapy.

Immunohistochemical analysis of the remaining 6 samples did not play a determinative role in making an accurate diagnosis. However, when evaluated together with resection material, interestingly, all of the immunoreactivity profiles were representative of the tumor. Among the 33 samples of our series where cell block and tissue compatibility was compared, a conformity rate of 94% was seen.

Monica et al.[23] observed TTF-1 expression in 35% of cell blocks of AC histological material demonstrating 80% TTF-1 positivity. They explained this condition with focal expression of TTF-1 in a tumor, which naturally precluded its detection in the cell block. In our study, TTF-1 positivity was demonstrated in 60% of cell blocks and in 68% of resection material. P63 and CK5/6 positivity, respectively, was detected in 73% and 64% of cell blocks, and in 91% and 73% of resection specimens. Immunoreactivity was not detected in any resection material of tumors where none of the markers demonstrated immunoreactivity in the cell blocks.

In conclusion, the results of our study indicated that the cytological sample taken from the tumoral mass defined the immune profile of the tumor to a great degree. Therefore, reserving some of the material obtained during FNAB for the preparation of cell blocks can lead to identification of morphological properties in the prepared slides, and this approach can aid in histological subtyping by using immunohistochemical markers, and also provide the necessary quantity of cells required for molecular studies to be performed in cases of AC.

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Akciğer Tümörlerinde Histolojik Alt Tiplendirme: Hedefe Yönelik Tedaviler ile Güncelleşen Bir Sorun

Amaç: İnce iğne aspirasyon örneklerinde akciğerin küçük hücreli dışı karsinomlarının histolojik alt tiplerini belirlemede sitomorfolojik karakteristiklerin ve immünhistokimyasal belirleyicilerin önemini araştırmak.

Gereç ve Yöntem: Arşiv kayıtlarından transtorasik ince iğne aspirasyonu ile küçük hücreli dışı karsinom tanısı alan ve cerrahi rezeksiyon uygulanan 73 hastaya ait örnekler çalışmaya dahil edildi. Sitolojik örnekler skuamöz hücreli karsinom ve adenokarsinom için tanımlanan sitomorfolojik özellikler açısından tekrar gözden geçirildi ve immünhistokimyasal incelemenin histolojik alt tiplendirmeye katkısı değerlendirildi.

Bulgular: Sitomorfolojik değerlendirmede skuamöz hücreli karsinom için keratinize lamellar sitoplazma, dens-hiperkromatik nükleus ve pleomorfik-poligonal hücrelerin; adenokarsinom için ise tek tabakalı örtüler ve nükleositoplazmik polaritenin %60 üzerinde sensitivite, spesifisite ve pozitif prediktif değere sahip olduğu gözlendi. İmmünhistokimyasal incelemede; TTF-1 antikorunun adenokarsinom için sensitivitesi %63, spesifisitesi %93; P63 ve CK5/6 antikorlarının skuamöz hücreli karsinom için sensitivite ve spesifisiteleri sırasıyla %73, %64 ve %82, %96 olarak gözlendi. Tümörün hücre blokları ve rezeksiyon materyallerinde gözlenen immünhistokimyasal uyumu %94 olarak belirlendi.

Sonuç: Akciğerin özellikle az diferansiye küçük hücreli dışı karsinomlarında immünhistokimyasal inceleme histolojik alt tiplendirmede yardımcı bir yöntemdir. Hücre bloğu tümörün immünprofilini yansıtan güvenilir bir tekniktir. Dolayısıyla aspiratın bir kısmının hücre bloğu hazırlanmak üzere ayrılması histolojik alt tiplendirmede yardımcı olurken, özellikle adenokarsinomlarda moleküler çalışmalar için gerekli materyali sağlayacaktır.

Anahtar Sözcükler: Histolojik alt tiplendirme; immünohistokimya; ince iğne aspirasyonu; küçük hücreli dışı akciğer kanseri; sitomorfoloji.