A comparative study of morphological and Immunohistochemical expression of P40 and P63 immunomarkers in squamous cell carcinoma and adenocarcinoma lung

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Abstract: *Background:* Lung cancer is a type of cancer that arises in the lungs. Adenocarcinoma and squamous cell carcinoma are the two major subtypes of non-small cell lung carcinoma. *Material and Methods:* This is a retrospective study conducted in the Department of Pathology, over a period of 1 year at a tertiary care hospital and teaching centre. A total of 125 cases comprising of 70 adenocarcinomas and 55 squamous cell carcinomas of lung were included in the study. *Result:* All 27 cases of well differentiated Adenocarcinoma were negative for P40 and 1 case showed positive P63 expression. Out of 43 cases of moderately differentiated Adenocarcinoma, 1 case was positive for P40 and 4 cases were positive for p63. Out of 19 cases of well differentiated Squamous cell carcinomas 18(94.7%) cases were positive for P40 and 18 (94.71%) cases were P63 positive. All 36 (100%) cases of moderately differentiated Squamous cell carcinoma showed positive P40 and P63 expression. *Conclusion:* Strong and diffuse P40 expression was noted in majority of lung squamous cell carcinomas and absence of P40 in most of the lung adenocarcinomas. Thus, p40 is an excellent marker for distinguishing lung squamous cell carcinoma from adenocarcinoma, and that its expression is equivalent to that of p63 in lung squamous cell carcinoma. Since P63 shows variable expression in lung adenocarcinomas; in moderately differentiated cases, a two-panel approach of p63 and p40 help to distinguish adenocarcinoma from squamous cell carcinomas.

Keyword: Immunomarker, Squamous cell carcinoma, Adenocarcinoma, Lung Carcinoma.

INTRODUCTION

Lung cancer is a type of cancer that arises in the lungs. Lungs are two spongy organs in the chest that take in oxygen while inhaling and release carbon dioxide while exhaling. Lung cancer is the leading cause of cancer deaths worldwide. [1] The number of deaths caused by lung cancer peaked at 159,292 in 2005 and has since decreased by 6.5 percent to 148,945 in 2016. [2] Adenocarcinoma and squamous cell carcinoma are the two major subtypes of non-small cell lung carcinoma. Until recently, therapeutic approaches to non-small cell lung carcinoma were largely guided by tumor stage, and there was no difference in treatment for adenocarcinoma *vs* squamous cell carcinoma. [2] This monolithic approach to non-small cell lung carcinoma has dramatically changed in the last few years as a result of three major advances in thoracic medical oncology for advanced disease. [3] Other important molecular differences between adenocarcinoma and squamous cell carcinoma are increasingly identified, suggesting that future targeted therapies will be increasingly 'histology specific.' [4] Selection of patients for appropriate molecular tests and histology-based therapies necessitates accurate pathologic distinction of adenocarcinoma *vs* squamous cell carcinoma. [5]

In most cases, the distinction of adenocarcinoma and squamous cell carcinoma is readily achieved based on standard morphologic criteria, with keratinization and intercellular bridges representing hallmarks of squamous cell carcinoma and glandular architecture (in the form of acini, papillae, micro papillae, or cytoplasmic mucin) representing the hallmarks of adenocarcinoma. [6] However, distinction can be difficult in some poorly differentiated tumors, where defining glandular or squamous features are subtle or focal. [7] This issue is particularly amplified in small specimens (small biopsies and cytology) where focal evidence of morphologic differentiation may not be represented as a result of scant cellularity, crush artifact, or cell dispersal. [8] Because ~70% of non-small cell lung carcinomas present at an unresectable stage, the only diagnostic material guiding systemic therapy in majority of such patients are small specimens. [9]

In parallel with the above clinical and molecular progress, the major advance in thoracic pathology in recent years has been the growing evidence that immunohistochemistry is a highly effective ancillary tool for distinguishing adenocarcinoma and squamous cell carcinoma. [10] Although the optimal diagnostic algorithm is not firmly established, recent studies show that immunohistochemistry increases accuracy and reproducibility, and minimizes the rate of non-small cell lung carcinoma—not otherwise specified diagnosis in small specimens. [11] Recent studies show that the rate of adenocarcinoma and squamous cell carcinoma unclassified by preoperative cytology in clinical practice with routine utilization of immunohistochemistry is low (3%). [12]

Immunohistochemistry (IHC) can be helpful in sub-classifying lung carcinomas on small biopsies. Commonly used markers include Napsin A and TTF-1 for adenocarcinoma and CK5/6, p63, and p40 for squamous cell carcinoma.

MATERIAL AND METHODS

This is a retrospective study conducted in the Department of Pathology, over a period of 1 year at a tertiary care hospital and teaching center.

Tissue samples

We analyzed 70 adenocarcinomas and 55 squamous cell carcinomas from the archives of pathology. Hematoxylin & eosin (H&E) stained slides were reviewed; paraffin blocks were used for immunohistochemical staining. Histopathological and clinical variables, including age, tumor size, differentiation, infiltrate depth, and lymph node metastasis, were noted.

Immunohistochemistry

Four to five micron-thick paraffin sections of the 125 cases were dewaxed, rehydrated in graded alcohols, and processed using the PV-9000 detection kit. Briefly, antigen retrieval was performed in a microwave oven for 3 min in 10 mM TrisEDTA buffer (10 mM Tris Base, 1 mM EDTA Solution, 0.05% Tween 20, pH 9.0). Endogenous peroxidase activity was blocked with a 1.7% H_2O_2-methanol solution for 30 min. Slides were then incubated in 10% normal goat serum for 30 min to prevent non-specific binding. Samples were then incubated overnight at 4 °C with a primary antibody. Phosphate Buffered Saline (PBS) was used instead of the first antibody as a negative control. Consequently, samples were incubated with Reagent 2 at room temperature for 30 min and Reagent 3 at room temperature for 20 min. Finally, the tissues were stained with diamino benzidine (DAB). Immunohistochemistry with p40 antibody was performed. Antigen retrieval was performed with CC1 buffer (Cell Conditioning 1; citrate buffer pH 6.0).

Immunohistochemistry for p63 (TP63; 4A4, Dako, 1:700 dilution) was performed. Whereas p40 recognizes an epitope which is unique to DNp63. For both markers, intensity (1+, 2+, and 3+) of immunoreactivity were recorded. Only nuclear immunoreactivity was considered as positive staining.

Statistical Analysis

Wherever applicable, descriptive statistical analysis was done.

RESULT

In the present study, a total of 125 patients were included out of which 89 (71.2%) were males and 36 (28.8%) were females (table-1).

Table 1: Distribution of gender

Gender	No. of patients	Percentage
Male	89	71.2
Female	36	28.8
Total	125	100

Table 2: Distribution of different age groups of patients

Age	No. of patients	Percentage
30-40 years	9	7.2
41-50 years	28	22.4
51-60 years	37	29.6
>61 years	51	40.8
Total	125	100

In our study, most of the patients were >61 years i.e., 51 out of 125 (40.8%), followed by 51-60 years, i.e., 37 out of 125 (29.6%).

Table 3: Morphological Grading of lung carcinomas (Broder's Grading) (n=125) (Grading of lung carcinoma)

Morphological Diagnosis	Grade/differentiation	No.	Percentage
Adenocarcinoma	Well differentiated	27	21.6
	Moderately differentiated	43	34.4
Squamous cell carcinoma	Well differentiated	19	15.2
	Moderately differentiated	36	28.8
Total		125	100

Table 4: Correlation of P63 Immunomarker expression with morphologic type of lung carcinoma (n =125).

Final Diagnosis		P63 Positive N (%)	P63 Negative N (%)	Total N
Adenocarcinoma	Well differentiated	1 (3.8)	26 (96.2)	27
	Moderately differentiated	4 (9.3)	39 (90.7)	43
Squamous cell carcinoma	Well differentiated	18 (94.7)	1 (5.3)	19
	Moderately differentiated	36 (100)	0 (0)	36
Total		59	66	125

Table 5: P63 intensity of staining in 59 positive cases (n=59)

Final Diagnosis		P63 Intensity 1+	P63 Intensity 2 +	P63 Intensity 3+	Total
Adenocarcinoma	Well differentiated	1	0	0	1
	Moderately differentiated	4	0	0	4
Squamous cell carcinoma	Well differentiated	0	8	10	18
	Moderately differentiated	0	15	21	36
Total		5	23	31	59

Table 6: Correlation of P40 Immunomarker expression with morphologic type of lung carcinoma. (n=125) (P40 immunomarker expression)

Final Diagnosis		P40 Positive N (%)	P40 Negative N (%)	Total N
Adenocarcinoma	Well differentiated	0 (0)	27 (100)	27
	Moderately differentiated	1 (2.3)	42 (97.7)	43
Squamous cell carcinoma	Well differentiated	18 (94.7)	1 (5.3)	19
	Moderately differentiated	36 (100)	0 (0)	36
Total		55	70	125

Table 7: P40 intensity of staining in 55 positive cases (n=55)

Final Diagnosis		P40 Intensity 1+	P40 Intensity 2 +	P40 Intensity 3+	Total
Adenocarcinoma	Well differentiated	0	0	0	0
	Moderately differentiated	1	0	0	1
Squamous cell carcinoma	Well differentiated	0	7	11	18
	Moderately differentiated	0	17	19	36
Total		1	24	30	55

Table 8: Concordance between morphological diagnosis and P40 and P63 IHC diagnosis (morphologic diagnosis and IHC diagnosis)

Final Diagnosis		P63	P40	Total
Adenocarcinoma	Well differentiated	1	0	27
	Moderately differentiated	4	1	43
Squamous cell carcinoma	Well differentiated	18	18	19
	Moderately differentiated	36	36	36

All 27 cases of well differentiated Adenocarcinoma were negative for P40 and 1 case showed P63 expression. Out of 43 cases of moderately differentiated Adenocarcinoma, 1 case was positive for P40 and 4 cases were positive for p63 marker. Out of 19 cases of well differentiated Squamous cell carcinoma 18 cases (94.7%) were P40 positive and 18 (94.71%) cases were P63 positive. All 36 cases of moderately differentiated Squamous cell carcinoma showed positive P40 and P63 expression.

DISCUSSION

Several recent studies have evaluated immunohistochemistry and special stain algorithms for non-small cell lung carcinoma subtyping. [13] Because of a large number of cases in this study, we were able to examine in greater detail the spectrum of possible expression profiles of commonly used markers in adenocarcinoma and squamous cell lung carcinomas, which serves as the basis for a more fine-tuned algorithm. Specifically, we show that the vast majority of specimens can be classified by p40 and p63. Whereas most prior studies agree on the inclusion of p40 and p63 in the diagnostic panels, various

other markers were included as well, whereas we clarify that p63 panel is sufficient to identify the line of differentiation in the majority of cases based on the proposed algorithm

P63 is a member of the p53 family, a classical tumor suppressor gene family. It is located on chromosome 3q27-29. Filho JS et al showed good sensitivity when detecting squamous cell carcinoma with a positive rate of 92.6%. [14] On contrary, Kaufmann et al suggested that P63 could also be expressed in a small number of adenocarcinoma, basal cell carcinoma, and transitional epithelial carcinoma. [15] Moreover, P63 can also be used as a marker for myoepithelial cells and prostate basal cells. Therefore, P63 lacks absolute specificity for squamous differentiation. [15]

P40 is a subtype of P63 protein expressed in squamous epithelial cells (including epidermis and hair follicles), urothelial cells, myoepithelial cells of the mammary gland, sweat gland, salivary gland and basal cells of the prostate, which are highly specific in labeling squamous epithelium. [16] Bishop et al showed that in 81 cases of squamous cell carcinoma of the lung and 237 cases of adenocarcinoma of the lung, the sensitivity and specificity of P63 were 100.00% and 69.20%, respectively. The sensitivity and specificity of P40 in the diagnosis of squamous cell carcinoma of the lung were 100% and 98%, respectively. [17] Therefore, P40 is considered as a highly specific and sensitive tumor biomarker of squamous epithelial origin. [18]

Similar to our findings, there is a general agreement in recent studies that classic profiles (p63-negative vs p63-positive) support the diagnosis of adenocarcinoma vs squamous cell carcinoma, respectively. These profiles are non-overlapping, and are not detected in the opposite tumor type in this or prior studies. [19]

Our study was conducted to investigate the expression of p40 and p63 in predominantly small biopsies and few resected specimens of lung squamous cell carcinoma and adenocarcinomas. The present study was in line with previous reports in concluding that p40 is more specific than the standard p63 marker for diagnosing lung squamous cell carcinoma.

CONCLUSION
We conclude that strong and diffuse p40 expression is seen in majority of lung squamous cell carcinomas and absence of p40 expression in most of the lung adenocarcinomas. Expression of p63 is similar to that of p40 in lung squamous cell carcinoma, but there was variable p63 immunoreactivity in lung adenocarcinoma. In Moderately differentiated cases, a two-panel approach of p63 and p40 help to distinguish adenocarcinoma from squamous cell carcinoma. Thus, p40 is an excellent marker for distinguishing lung squamous cell carcinoma from adenocarcinoma and that its expression is equivalent to that of p63 in lung squamous cell carcinoma.

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