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Original Research Article

A Comparative Analysis of P40 and P63 Immunohistochemical Markers in Differentiating Squamous Cell Carcinoma and Adenocarcinoma of Lung

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Abstract

Background: Lung cancer is the leading cause of cancer related deaths worldwide and accounts for 28% of all cancer mortality. Several histopathological types of lung cancer exist, among them the majority are non-small cell carcinomas (NSCLCs) including adenocarcinoma (ADC) and squamous cell carcinoma (SqCC). In light of discovery of molecular alterations associated with lung ADCs and advent of targeted therapies, further subtyping of NSCLCs has profound therapeutic implications.

Materials and Methods: A total number of 90 consecutive cases of NSCLCs diagnosed on core-needle biopsies (CNBs) and endobronchial biopsies (EBBs) of the lung sent for histopathological evaluation were included in the study. The demographic data, clinical details, radiological features, and laboratory investigations were retrieved. The Small cell lung carcinomas (SCLC), carcinoids, lymphomas, mesenchymal neoplasms, metastatic carcinomas, and mesotheliomas were not included in the study.

Result: Out of 90 cases 60 were ADC and 30 were SqCC. Most of the patients were in the age group of 61-70 years, 10 were between 71-80 years, 10 were between 51-60years and 10 were between 40-50 years. In this study the sensitivity and the specificity of p63 were 100% and 80% respectively, and sensitivity and specificity of p40 were 100% and 98.3% respectively. Positive predictive value was higher for the p40 compared to p63. To summarize, sensitivity of p63 and p40 was found to be the same, but the specificity and positive predictive value were higher for p40 for diagnosis of SqCC. One case of ADC showed positivity for p40 which may be due to adeno-squamous carcinoma misdiagnosed as ADC on cytology.

Conclusion: P63 has a better sensitivity, and P40 has a better specificity for SqCC. A positive staining pattern with both markers was also found in certain non-SqCC cases.

Keywords: Non-small cell lung carcinoma (NSCLC); Immunohistochemical markers; P40 and P63.

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Introduction

Lung cancer is the leading cause of cancer related deaths worldwide and accounts for 28% of all cancer mortality. [1] In India, lung cancer constitutes 6.9 percent of all new cancer cases and 9.3 percent of all cancer related deaths. [2] Several histotypes of lung cancer exist, among them the majority are non-small cell carcinomas (NSCLCs) including adenocarcinoma (ADC) and squamous cell carcinoma (SqCC). In light of discovery of molecular alterations associated with lung ADCs and advent of targeted therapies, further subtyping of NSCLCs has profound therapeutic implications. [3] In most cases, the distinction of ADC and SqCC are readily achieved based on standard morphologic criteria, with tumour cells showing keratinization and intercellular bridges representing hallmarks of SqCC and glandular architecture (in the form of acini, papillae, micropapillae, or cytoplasmic mucin) representing the hallmarks of ADC. [4] However differentiation of lung ADCs from SqCCs may often be difficult to achieve based on histomorphology alone, especially in poorly differentiated tumors. [5] This issue is particularly amplified in small specimens where focal evidence of morphologic differen tiation may not be represented as a result of scant cellularity, crush artifact, or cell dispersal and may require a panel of immunohistochemistry (IHC) markers. [6] In order to meet the above challenge, IHC has been shown to be a valuable adjunct to H&E staining, particularly for poorly differentiated/ undifferentiated tumors. [7-10] To make more effective use of limited tissue, multiplex IHC approaches have been developed, wherein two or more antibodies directed against morphologically distinct antigens are added to the same tissue sample. Each antibody can be detected using a different color chromogen. [11] Several studies

have shown that p63 has an extremely high sensitivity for SqCC. [12-16] However; studies using antibodies against p63 alone have demonstrated false positive results with positivity in some ADC. Another important limitation of p63 as a 'squamous marker' is its unexpected expression in several other tumors, particularly lymphomas, where reactivity has been reported in up to half of the cases. [15]

The anti-p40 antibody, a relatively new immunomarker, has been reported in several studies for the distinction of lung SqCC and ADC, suggesting that unlike p63 antibody, p40 antibody is highly squamous-specific. [16] And thus the anti-p40 antibody has been recommended instead of the anti-p63 antibody for the diagnosis of pulmonary SqCC. The present study aims at evaluation of p40, a relatively new immunomarker, and compares its sensitivity and specificity with p63, a commonly used immunomarker in subtyping of NSCLC into SqCC and ADC.

Materials and Methods

This prospective study was conducted on a total number of 90 consecutive cases of NSCC diagnosed on core-needle biopsies (CNBs), endobronchial biopsies (EBBs) of the lung sent for histopathological evaluation. The demographic data, clinical details, radiological features, and laboratory investigations were retrieved. The Small cell lung carcinomas, carcinoids, lymphomas, mesenchymal neoplasms, metastatic carcinomas, and mesotheliomas were not included in the study.

Morphological Analysis: Initially, all the cases of NSCC were morphologically categorized based on the review of H and E slides blinded to the results of special stains and IHC results. Subsequently, special stains with Alcian blue and Periodic acid–Schiff for mucin if performed were reviewed.

Immunohistochemistry: In all cases where IHC was done, the slides were reviewed to subtype the NSCC. In those cases where IHC was not done, the initial morphological diagnosis was considered as a final diagnosis. The primary panel of SqCC and ADC markers included p40, p63, CK5/6, TTF1, Napsin-A, and CK7.

All cases of NSCC were categorized as per the proposed International Association for the Study of Lung Cancer American Thoracic Society European Respiratory Society classification for small biopsies. [2,3] For all the markers, the intensity of staining was taken into consideration and was compared to positive controls. For the SqCC markers, p40 and p63, nuclear staining was accepted, and cytoplasmic staining was ignored. For p40 and p63 antibody, the intensity of staining was scored semi-quantitatively using a 3-tier system: weakly positive (Grade 1), moderate positivity (Grade 2), and strongly positive (Grade 3). The staining proportion pattern was scored on a 4-tier system: <5%; 6%–25%; 26%–50%; and >50%. Cases showing positivity of 5% or less were considered negative.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of both p40 and p63 markers in diagnosing ADC and SqCC markers were analyzed.

Results

During the study period, 90 NSCLC were diagnosed and out of them 60 cases were diagnosed as ADC and 30 cases were diagnosed as SqCC. Most of the patients were in the age group of 61-70 years which consisted 60 cases.10 cases in the age group of 71-80 years, 10 cases between the age group of 51-60 years and 10 cases between the age group of 40-50 years. When analyzed for the gender distribution, we observed that male preponderance was observed with a M:F ratio of 3:1. The immunoreactivity for p63 vs p40 in SqCC and ADC are described in Table 1 and the sensitivity and specificity of p63 and p40 are described in Table 2. The positive and negative predictive value is elaborated in Table 3.

The sensitivity and the specificity of p63 were 100% and 80% respectively, and sensitivity and specificity of p40 were 100% and 98.3% respectively. Positive predictive value was found to be higher for the p40 when compared to p63. To summarize, sensitivity of p63 and p40 were found to be same, but the specificity and positive predictive value were higher for p40 for diagnosis of SCC. One case of ADC showed positivity for p40 which may be due to adeno-squamous differentiation. We have illustrated a few of them in detail, figure 1 showing SqCC histopathology H and E (40x) with sheets and clusters of atypical squamous cells with moderate to abundant eosinophilic cytoplasm and pleomorphic hyperchromatic nucleus some of them having vesicular nucleus with prominent nucleoli. Staining pattern of both p63 and p40 are also show in in images b and c.

Table 1: Immuno-reactivity for p63 vs p40 in SCC and ADC

Tumor type	Antibody	Number of cases	Positive	Negative
	P63	30	30	0
SCC	P40	30	30	0
	P63	60	15	45
ADC	P40	60	1	59

Table 2: Sensitivity and specificity of p63 and p40

Marker	SCC	ADC	Sensitivity	Specificity
P63	30/30	15/60	100	60
P40	30/30	01/60	100	98.3

Table 3: Positive and negative predictive value

Marker	SCC	ADC	Positive predictive value	Negative predictive value
P63	30/30	15/60	75	100
P40	30/30	01/60	97.3	100

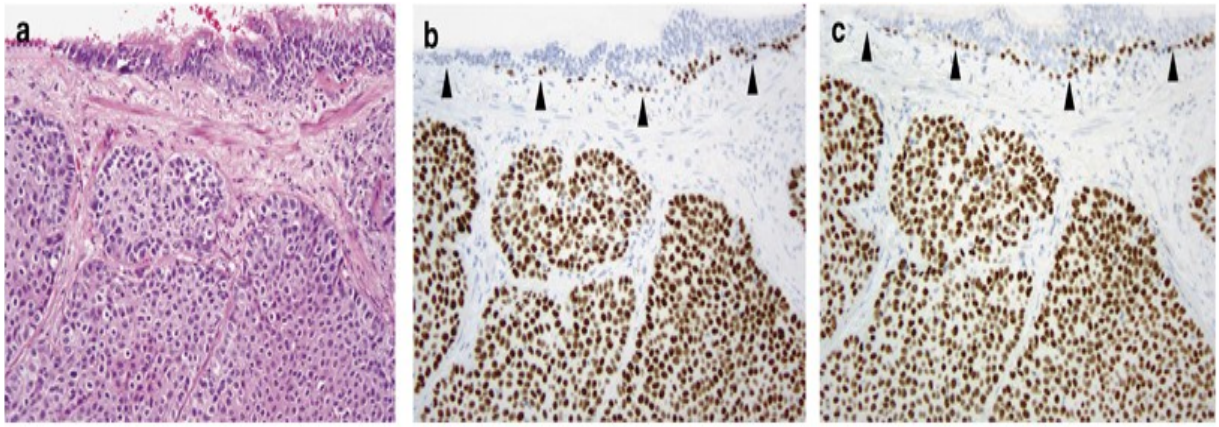

Figure 1: p63 versus p40 reactivity in squamous cell carcinoma. (a–c) This poorly differentiated squamous cell carcinoma does not have overt squamous differentiation in this field (a, hematoxylin and eosin, × 200), but p63 (b, p63 immunohistochemistry, × 200), and p40 (c, p40 immunohistochemistry, × 200) are strongly and diffusely positive.

Discussion

The World Health Organization (WHO) lung classification system lists the following major subcategories for malignant epithelial tumors: squamous cell carcinoma, adenocarcinoma, large cell carcinoma, adenosquamous carcinoma, sarcomatoid carcinoma, carcinoid tumor, salivary gland tumors and unclassified carcinoma.

NSCLC (which is mainly comprised of SqCC and ADC) accounts for approximately 80% of all lung cancers. [20,21] From therapeutic standpoint, NSCLC has been subdivided into either SqCC or non-SqCC—including ADC, large cell carcinoma, and poorly differentiated carcinoma (PDCA), which is also known as NSCLC not otherwise specified (NOS). EGFR and ALK testing is recommended for the NSCLC, particularly in the last three categories where, if positive, tyrosine kinase inhibitors or ALK-inhibitors are included in therapeutic regimens. [22]

Several studies have shown that p63 has an extremely high sensitivity (approaching 100%) for SqCC.[20] A study by Pelosi et al found that p63 immuno-reactivity was seen in 109/118 SqCCs, 15/95 ADCs, 2/2 adenosquamous carcinomas, 4/6 large cell carcinomas, 9/20 poorly differentiated NET, and 1/37 typical and atypical carcinoids, which indicate that its quite variable.[23] However, the main limitation of p63 is low specificity due to its unexpected reactivity in 16-65% of lung ADCs even on the cytology smears. [24] Therefore, there was a need felt for alternative marker for the SCC lung, which was found in the form of p40. A study by Affandi et al found that p40 is an excellent marker for distinguishing lung SqCC from ADC, and p40 expression is equivalent to p63 expression in lung SqCC. [25] In the present study we did a detailed evaluation of the expression of p63 and p40 in various NSCLC.

The present study sample size, study duration, age and the gender distribution are comparable to the published literature, where the age range is around 5-7 th decade with similar M:F ratio. Similarly, the percentage of SqCC and ADC were also comparable, making our study results concurrent with published literature which has been elaborated in table 4. [26] When we compared the sensitivity and specificity of the present study with the published literature, we found that sensitivity of p63 and p40 were equal in making the diagnosis of SqCC lung. However, specificity for p40 was higher for the diagnosis. The sensitivity of the parent study is compared with other similar studies in table 5. [27] p40 has been reported to have better sensitivity and specificity than p63 in the identification of SqCCs. Interestingly, recent studies have shown p40 immunostaining in benign and malignant neoplasms other than SqCC. A recent study has demonstrated the role of p40 as a

marker for sebaceous differentiation, noting that this antibody can be utilized for diagnosing sebaceous carcinoma in the setting of poorly differentiated carcinoma. [28] p40 is also expressed in the cuboidal tumor cells of sclerosing hemangioma of the lung, but not in the polygonal tumor cells. [29]In the current pathology practice p40 has become a valuable marker for SqCC, especially when encountered with poorly differentiated NSCLC. [30]

Table 4: Comparison of present study with existing literature

Study	Number of cases	Age range (in years)	Duration	Squamous vs adeno
Present study	90	40-80	3 years	30:60
Lilo et al [25]	114	33-88	4 years	14:100
Delgado et al [26]	56	46-88	7 years	14:42
Vogt et al [27]	40	42-81	5 years	20:20
Bishop et al [28]	298	40-85	4 years	71:227

Table 5: Comparison of sensitivity and specificity of p63/ p40 in SCC

Study	P63		P40	
	Sensitivity	Specificity	Sensitivity	Specificity
Present study	100	60	100	98.3
Vogt et al [27]	97	60	100	100
Righi et al [29]	95	60	100	95
Lilo et al [25]	79-100	30-65	66.4-90.1	80.6-100
Delgado et al [26]	62	72	100	100
Bishop et al [28]	100	60	100	95

Table 6: Comparison positive and negative predictive value of p63 in SCC

Study	P63		P40	
	Positive predictive value	Negative predictive value	Positive predictive value	Positive predictive value
Present study	72.6	97.5	96.4	100
Vogt et al [27]	60	96	100	100
Righi et al [29]	60	96	90	100
Delgado et al [26]	75	90	100	100
Bishop et al [28]	30	100	90	100

Conclusion

Although the commonly used squamous cell marker p63 is extremely sensitive, it is less specific due to its reactivity in few cases of ADC. A potentially useful new marker p40, an isoform of p63, is equally sensitive and more specific than p63 for diagnosis of SqCC and is negative in ADC.

Positive p63 staining may be mistakenly interpreted as squamous differentiation and result in misclassification of ADC as squamous cell lung carcinoma. Therefore p40 can be used instead of p63 as a squamous cell marker..

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