IAR Journal of Medicine and Surgery Research
ISSN Print: 2709-1899 | **ISSN Online:** 2709-1902
Frequency: Bi-Monthly
Language: English
Origin: KENYA
Website: https://jmsrp.or.ke/index.php/jmsrp

OPEN ACCESS JOURNALS

Research Article

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

Article History

Received: 12.01.2024
Revision: 17.01.2024
Accepted: 05.02.2024
Published: 28.02.2024
Plagiarism check - Plagscan
DOI: 10.47310/iarjmsr. 2024.V05i01.03

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How to Cite the Article:
Shalini R, Ramakrishna KR. A comparative study of morphological and Immunohistochemical expression of P40 and P63 immunomarkers in squamous cell carcinoma and adenocarcinoma lung. *IAR J. Med & Surg Res. 2024;5(1):*11-15.

Abstract: ***Background:*** Lung cancer is the leading cause of cancer related death worldwide and accounts for 28% of all cancer mortality and around 1.8 million new cases were diagnosed in 2012. The morphological distinction between pulmonary adenocarcinoma (ADC) and squamous cell carcinomas (SCC) is sometimes difficult, mainly in cases of poorly differentiated tumors or when degenerative changes, necrosis and crushing may obscure the cell characteristics. p63 is a homologue of the p53 tumour suppressor gene that is responsible for proliferation and differentiation of epithelial progenitor cells. p40 is consistently the predominant isoform expressed in squamous cell carcinoma; thus, it offers improved specificity for diagnosing squamous cell carcinoma. ***Material and Methods***: This is a prospective and observational study conducted in the Department of Pathology, Tertiary care Teaching Hospital over a period of 1 year. Primary lung carcinoma cases included with unequivocal morphological diagnosis irrespective of age, gender and nature of biopsy material (endoscopic biopsy/ needle core biopsy / resected specimen). Cases diagnosed as Small cell carcinoma of lung, as metastatic lung cancers, poorly differentiated NSCLC-NOS and with inadequate material for IHC study were excluded from present study. ***Results****:* In the present study, a total of 150 patients were included out of which 112 (74.6%) were males and 38 (25.4%) were females. In our study, most of the patients were >61 years i.e., 63 out of 150 (42%), followed by 51-60 years, i.e., 40 out of 150 (26.7%). All 8 cases of well differentiated Adenocarcinoma were positive for P40 and 5 cases showed P63 expression. Out of 13 cases of moderately differentiated Adenocarcinoma, 3 cases were positive for P40 and 10 cases were positive for p63 marker. Out of 30 cases of well differentiated Squamous cell carcinoma 15 cases were P40 positive and 15 cases were P63 positive. All 53 cases of moderately differentiated Squamous cell carcinoma showed positive P40 and P63 expression.

Keywords: Morphological and Immunohistochemical, P40 and P63, carcinoma nad Adenocarcinoma

INTRODUCTION

Lung cancer is the leading cause of cancer related death worldwide and accounts for 28% of all cancer mortality and around 1.8 million new cases were diagnosed in 2012. [1] In India, lung cancer constitutes 6.9 percent of all new cancer cases and 9.3 percent of all cancer related deaths. [2] [5] Identifying the correct type of lung cancer has become increasingly important due to the recent advances in "targeted" therapies. The morphological distinction between pulmonary adenocarcinoma (ADC) and squamous cell carcinomas (SCC) is sometimes difficult, mainly in cases of poorly differentiated tumors or when degenerative changes, necrosis and crushing may obscure the cell characteristics. [3]

Poorly differentiated non-small-cell lung carcinomas (PD-NSCLC) are tumors characterized by predominantly solid growth, formed by cells with large, eosinophilic cytoplasm, with a misleading tendency to keratinization or a pseudosquamoid morphology. [4] Thus, histochemical detection of mucin and immunohistochemistry are often essential tests in differential diagnosis of NSCLCs, expanding the routine morphological examination. [5]

p63 is a homologue of the p53 tumour suppressor gene that is responsible for proliferation and differentiation of epithelial progenitor cells. The p53 gene contains two promoters that produce two isoforms; one isoform contains the N-terminal transactivation domain (TAp63) and the other lacks this domain (ΔNp63). [6]

p63 is normally expressed in the nuclei of basal and progenitor cells of stratified epithelia such as skin, esophagus, tonsil, urothelium, ectocervix, and vagina, and in the basal cells of glandular structures of the thymus, prostate, breast, and bronchi [7]. Both TAp63 and ΔNp63 show overlapping distribution in some epithelial tissue. However, TAp63 is more expressed in differentiated cells while ΔNp63 is seen in the stem-like cell populations [8].

Antibody p40, which identifies ΔNp63, has been available for several years but its use for distinction of lung squamous cell carcinoma and adenocarcinoma was only recently studied. p40 is consistently the predominant isoform expressed in squamous cell carcinoma; thus, it offers improved specificity for diagnosing squamous cell carcinoma. Studies by Bishop et al. and Nonaka [9] showed that p40 has 100% sensitivity and specificity in lung squamous cell carcinoma. Another study by Tacha et al. reported an 85% sensitivity and 98% specificity. [10]

Material and Method

This is a prospective and observational study conducted in the Department of Pathology, Tertiary care Teaching Hospital over a period of 1 year.

Inclusion Criteria:

Primary lung carcinoma cases included with unequivocal morphological diagnosis irrespective of age, gender and nature of biopsy material (endoscopic biopsy/ needle core biopsy / resected specimen)..

Exclusion Criteria:

1. Cases diagnosed as Small cell carcinoma of lung, as metastatic lung cancers, poorly differentiated NSCLC-NOS and with inadequate material for IHC study were excluded from present study.
2. Cases were diagnosed by Hematoxylin (Meyer's) and Eosin stain. The grading were rendered on the basis of broder's grading system and classified into well and moderately differentiated SqCC and ADC.
3. IHC Analysis were done by using antigen retrieval method: BIO GENEX-EZRetriever system V.2 (temperature controlled microwaving). Percentage of SqCC and ADC showing positivity for P40 and P63 immunomarker and Sensitivity and specificity was calculated. Immunohistochemistry with p40 antibody was performed. Antigen retrieval was performed with CC1 buffer (Cell Conditioning 1; citrate buffer pH 6.0, Ventana Medical Systems). Immunohistochemistry for p63 (TP63; 4A4, Dako, 1:700 dilution) was performed. P63 (4A4) recognizes an epitope shared by TAp63 and DNp63 isoforms, whereas p40 recognizes an epitope which is unique to DNp63. For all markers, both extent (% cells) and intensity (1+, 2+, and 3+) of immunoreactivity were recorded. Only nuclear immunoreactivity was accepted.
4. Cases of poorly differentiated non-small cell (large cell) carcinomas where the tumor predominantly showed solid growth with no apparent squamous or glandular differentiation, and the diagnosis of poorly differentiated (large cell) carcinoma was made and final diagnosis was suggested purely on the basis of immunohistochemistry findings: that is, TTF-1, p63 and p40 expression.

Results

In the present study, a total of 150 patients were included out of which 112 (74.6%) were males and 38 (25.4%) were females (table-1).

Table 1: Distribution of Gender

Gender	No. of patients	Percentage
Male	112	74.6
Female	38	25.4
Total	150	100

Table 2: Distribution of different age groups of patients

Age	No. of patients	Percentage
30-40 years	15	10
41-50 years	32	21.3
51-60 years	40	26.7
>61 years	63	42
Total	150	100

In our study, most of the patients were >61 years i.e., 63 out of 150 (42%), followed by 51-60 years, i.e., 40 out of 150 (26.7%).

Table 3: Morphological Grading of lung carcinomas (Broder's Grading) (n=150) (Grading of lung carcinoma)

Morphological Diagnosis	Grade/differentiation	No.	Percentage
Adenocarcinoma	Well differentiated	30	20
	Moderately differentiated	45	30
Squamous cell carcinoma	Well differentiated	35	23.3
	Moderately differentiated	40	26.7
Total		150	100

Table 4: Correlation of P63 Immunomarker expression with morphologic type of lung carcinoma (n =150).

Final Diagnosis		P63 Positive N (%)	P63 Negative N (%)	Total N
Adenocarcinoma	Well differentiated	5 (3.3)	25 (16.7)	30
	Moderately differentiated	10 (6.7)	35 (23.3)	45
Squamous cell carcinoma	Well differentiated	17 (11.3)	18 (12)	35
	Moderately differentiated	15 (10)	25 (16.7)	40
Total		47	103	150

Table 5: Distribution of Antibiotic Resistance Data of 59 E.coli islate

Final Diagnosis		P63 Intensity 1+	P63 Intensity 2+	P63 Intensity 3+	Total
Adenocarcinoma	Well differentiated	1	2	2	5
	Moderately differentiated	4	3	3	10
Squamous cell carcinoma	Well differentiated	0	7	10	17
	Moderately differentiated	0	7	8	15
Total		5	19	23	47

Table 6: Correlation of P40 Immunomarker expression with morphologic type of lung carcinoma. (n=150) (P40 immunomarker expression)

Final Diagnosis		P40 Positive N (%)	P40 Negative N (%)	Total N
Adenocarcinoma	Well differentiated	3 (2)	27 (18)	30
	Moderately	3 (2)	42 (28)	45
	differentiated			
Squamous cell carcinoma	Well differentiated	15 (10)	20 (13.3)	35
	Moderately differentiated	36 (24)	4 (2.7)	40
Total		57	93	150

Table 7: P40 intensity of staining in 60 positive cases (n=57)

Final Diagnosis		P40 Intensity 1+	P40 Intensity 2+	P40 Intensity 3+	Total
Adenocarcinoma	Well differentiated	3	0	0	3
	Moderately differentiated	3	0	0	3
Squamous cell carcinoma	Well differentiated	0	5	10	15
	Moderately differentiated	0	17	18	36
Total		6	22	28	57

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	5	3	8
	Moderately differentiated	10	3	13
Squamous cell carcinoma	Well differentiated	15	15	30
	Moderately differentiated	17	36	53

All 8 cases of well differentiated Adenocarcinoma were positive for P40 and 5 cases showed P63 expression. Out of 13 cases of moderately differentiated Adenocarcinoma, 3 cases were positive for P40 and 10 cases were positive for p63 marker. Out of 30 cases of well differentiated Squamous cell carcinoma 15 cases were P40 positive and 15 cases were P63 positive. All 53 cases of moderately differentiated Squamous cell carcinoma showed positive P40 and P63 expression.

Discussion

Accurate histologic diagnosis and subtyping has major therapeutic implications. Current clinical practice guidelines recommend patients with lung adenocarcinoma or NSCLC favouring adenocarcinoma should undergo molecular testing. To our knowledge, our study is the first to present data on changes in categorization of resected pulmonary NSCC using the new WHO classification. In line with the IASLC/ATS/ERS proposal for small specimens, the fourth edition of the WHO classification introduces IHC markers of SCC and AC on resected lung tumors. [11]

Although the morphological findings are clear in most cases, IHC staining now defines many former LCC cases as AC or SCC and also helps to identify so-called pseudosquamous AC (accounting for approximately 1% of the AC cases) and what may correspondingly be called pseudo-adenocarcinomatous or "pseudoglandular" SCC. The changes in classification mainly reduced the LCC and ASqC groups; the classification of AC and SCC were only marginally affected. [12] Consequently, the modifications in the fourth WHO classification clearly help pathologists to better define previously difficult diagnostic groups without deforming well-established histological classification. [13]

However, a change in classification does not always lead to an altered therapeutic strategy. In only 12 of the cases (1.8%) in the present study (one, three, and three cases of AC, LC, and ASqC, respectively, now assigned to the SCC group and five cases of SCC now assigned to the AC group) the revised histological diagnosis would imply an altered clinical management in a potential later treatment situation. [14]

In our opinion, diagnostic morphological features may be more challenging to assess than is IHC expression in poorly differentiated cases and in cases with technical artefacts due to poor formaldehyde fixation. For example, identification of intercellular bridges in the absence of keratinization and differentiation of apoptotic cells from unicellular keratinization may be challenging. [15] Thus, differences in diagnoses may be related not only to changes in the WHO classification but also to the experience of the diagnosing pathologist, thus affecting the results of the present study as well. [16] Although different technical factors may affect the results of IHC staining, we believe that the increased emphasis on IHC staining in the fourth edition of the WHO classification results in a more reproducible histological classification of resected pulmonary NSCCs (although not examined in the present study). [17]

In the present investigation, there was a substantial overlap between p63 and p40, the markers recommended for SCC diagnosis. Adding p63 yielded limited gain, and its low specificity for SCC makes it a less appealing marker. Correspondingly, the results of the recommended markers for a diagnosis of AC, TTF-1 and napsin A, also overlapped substantially, although it is noteworthy that 13 of 79 solid predominant AC cases were positive for TTF-1 and negative for napsin A. [18] However, the results of staining with the TTF-1 clone SPT24 were also positive in approximately 5% of SCC cases, which may be a diagnostic problem if strictly following the guideline that a (poorly differentiated) NSCC be classified as AC if the result for an AC marker is positive regardless of any expression of SCC markers. [19]

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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