ISSN: 0975-1556

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2021; 13(5); 626-632
Original Research Article

A Study of Immunohistochemical Markers P63 and TTF-1 for Lung Pathologies

Anil Mohan Rao S

Assistant Professor, Department of Pathology, Pratima Institute of Medical Sciences Karimnagar, Telangana State, India.

Received: 01-09-2021 / Revised: 21-09-2021 / Accepted: 20-10-2021
Corresponding author: Dr. Anil Mohan Rao S
Conflict of interest: Nil

Abstract

Background: Biopsies of the lungs are crucial for detecting cancer. The fact that a lung biopsy only provides a little amount of tissue means that we only need to perform a small number of reliable tests. Only a small portion of the diagnostic process involves immunohistochemistry. P63 and TTF-1 have both been proposed as squamous cell carcinoma and adenocarcinoma indicators. The present study aimed to evaluate Immunohistochemical markers in biopsies of the lung using TTF-1 and p63.

Methods: A total of n=30 lung samples were examined histopathologically. The tumors were then immunohistochemically evaluated using the two markers TTF-1 and p63 once they were proven to be malignant. The cases were classified using the 2011 International Association for the Study of Lung Cancer/ American Thoracic Society/ European Respiratory Society (IASLC/ATS/ERS) classification.

Results: The number of Non-Small Cell Lung Carcinoma Not Otherwise Specified cases reported by H & E were 24.24% by IHC and were reduced to 9.09%. Out of the total n=5 cases the diagnosis following staining by TTF-1 and p63 all the n=5 cases were diagnosed as Non-Small Cell Lung Carcinoma in favor of Adenocarcinoma because they were found to be positive for TTF-1 and negative for p63. To summarize the most important result from the IHC was the diagnoses that were derived from the previously grouped NSCLC NOS. There was a 15.15% reduction of cases from this group with n= cases getting a clearer diagnosis. The remaining n=2 cases were found to be negative for both TTF-1 and p63 and could not be subclassified.

Conclusion: TTF-1 and P63 can be considered reliable indicators for further sub-tying of non-small cell lung cancer. TTF-1 and P63 IHC are useful for distinguishing between adenocarcinoma and squamous cell carcinoma, which is significant given that novel medicines have been developed with varying therapeutic effects depending on the histologic type.

Keywords: Lung biopsy, Bronchoscopy, Immunohistochemical markers, Thyroid transcription factor (TTF-1), p63.

Introduction

Lung cancer is the most common cause of cancer worldwide, diagnosed at the rate of 11.4% of all cancers reported in 2020. [1] Lung cancer was less common in the early part of the twentieth century than liver, prostate, colon, stomach, breast, and even

ovarian cancer. Non-small cell lung cancers (NSCLC) account for about 80% of lung carcinomas, while small cell lung cancers (SCLC). account for 20%. Squamous cell carcinomas are the most prevalent histological kind in the world. Smoking is linked to all histological kinds of lung cancer, but the association is stronger for squamous cell carcinomas and small cell carcinomas than for adenocarcinomas. It is impossible to overstate the importance of distinguishing between distinct lung cancers. Squamous cell carcinoma and adenocarcinoma of the lung, for example, have quite different therapy options. Many times, resecting a lung tumor is difficult, and in any instance, a diagnosis is essential before treatment can begin. This can be challenging since certain cases lack distinction or lack morphology that allows us to make a diagnosis. This is where immunohistochemistry comes in; it can help us figure out the nature of pathology involved in the lesion. Thyroid transcription factor (TTF-1, thyroid-specific enhancer-binding protein) is the single most important target for the Immunohistochemical study of pulmonary pathology. TTF-1 expression in lung tumors: The rate of positivity differs across the variety of lung tumors, broadly, 84% of small cell carcinomas, 77% of adenocarcinomas but only 8% of squamous cell carcinomas are positive for TTF-1. [2, 3] p63 is a member of the p53 tumor suppressor gene family. [4] The gene is located on chromosome 3q27-20. p63 is expressed predominantly in basal cell and squamous cell carcinomas, as well as transitional cell carcinomas, but not in adenocarcinomas (neither in primary nor in metastatic), large cell carcinomas, or small cell carcinomas. TTF-1 is regarded as the single most important immunohistochemical marker in the research of lung malignancies, and P63 estimation has been utilized to distinguish between primary lung tumors. P63 is a sensitive squamous cell carcinoma marker. [5] whereas TTF-1 has been demonstrated to be a sensitive adenocarcinoma marker. Adenocarcinomas and neuroendocrine carcinomas, such as small cell carcinoma, do not frequently stain with p63. Small cell carcinomas are stained by TTF-1, whereas metastatic lesions and squamous cell carcinomas are not. Misclassifications of tumors can be avoided if these markers are used correctly. As a result, the patient may profit greatly. TTF-1 and p63 are both nuclear proteins, which eliminates the difficulties associated with detecting cytoplasmic markers in tumor cells with minimal cytoplasm. [6] In light of the above facts, we in the present study aimed to evaluate Immunohistochemical markers in biopsies of the lung using TTF-1 and p63.

Material and methods

This cross-sectional study was conducted in the Department of Pathology, Prathima Institute of Medical Sciences, Naganoor, Karimnagar, Telangana State. Institutional Ethical approval was obtained for the study. Written consent was obtained from all the participants of the study. N=30 consecutive bronchoscopic and CT-guided biopsies obtained in the Department of Pathology were taken for the study.

Inclusion criteria

1. Successive bronchoscopic and CT guided biopsies
2. Suspected of lung pathologies
3. Received by the Department of Pathology, PIMS, Karimnagar

Exclusion criteria

1. Biopsies of patients already diagnosed with tumors and on treatment
2. Biopsies that were inconclusive for malignancies
3. Samples of biopsies that were improperly fixed

Tissues were fixed in a 10% neutral buffered formalin solution and processed as usual. Tissues were sliced 4 to 6 microns thick and stained with hematoxylin and eosin as usual. Two pathologists examined the slides and

determined the diagnosis based on the 2011 IASLC/ATS/ERS (International Association for the Study of Lung Cancers/ American Thoracic Society/ European Respiratory Society) guidelines for lung biopsy specimens. [7] This preliminary report was based on routine hematoxylin and eosin staining and was diagnosed as squamous cell carcinoma, adenocarcinoma, Non-small cell lung carcinoma (NSCLC) favor squamous cell carcinoma, NSCLC favor adenocarcinoma, NSCLC NOS (not otherwise specified), small cell lung carcinoma (SCLC), and one case of Large Cell Neuroendocrine Carcinoma (LCNEC). TTF-1 and p63 immunohistochemical markers were used to stain all of these confirmed cancers. The marker synaptophysin was used to confirm tumors having neuroendocrine differentiation, such as small cell carcinomas and large cell neuroendocrine carcinomas. Following the completion of immunohistochemistry, the sections were inspected microscopically for antibody localization and intensity. When the synaptophysin stain was utilized, a positive result was defined as unmistakable nuclear staining of at least 10% of the targeted tumor cells for both TTF-1 and p63, as well as cytoplasmic staining. Follicular carcinoma of the thyroid was employed as a TTF-1 control. A skin biopsy was used as a control for p63.

Results

Out of the total n=33 cases included in study n=25(75.75%) were males and n=8(24.24%) were females. The male to female ratio was approximately 3:1. The mean age of the males in the study was 48.56 ± 9.54 years and similarly, the mean age of the females in the study was 51.25 ± 5.4 years. The youngest case was 35 years old male and the oldest was 73 years female. The demographic distribution of cases is depicted in table 1.

Table 1: Age and Sex wise distribution of cases in the study

Age group in years	*Males (%)*	*Females (%)*	*Total of Cases (%)*
31 - 40	03 (12.0)	0 (0.00)	3 (9.09)
41 - 50	05 (20.0)	1 (12.5)	6 (18.18)
51 - 60	08 (32.0)	4 (50.0)	12 (36.36)
61 - 70	07 (28.0)	2 (25.0)	9 (25.0)
71 - 80	02 (8.00)	1 (12.5)	3 (9.09)
Total	25 (100.00)	8 (100.0)	33 (100.0)

Following routine staining, by H&E the biopsies were reported as indicated in the table above (Table 2). The most common interpretation was non-small cell lung cancer (NSCLC) in 33.33% of cases, and Non-Small Cell Lung Carcinoma Not Otherwise Specified in 24.24% of cases. After this the diagnosis of adenocarcinoma was done in 15.15% of cases, followed by small cell carcinomas with 4 cases (10%). Next, the diagnosis of small cell carcinoma and squamous cell carcinoma was in 9.09% of cases. 6.06% of cases were diagnosed as Non-Small Cell Lung Carcinoma in favor of Adenocarcinoma. And one case was diagnosed as a Large Cell Neuroendocrine Tumor. There was a total of n=8 women in this study, this was 24.24% of the cases. N=2 of them had adenocarcinoma, N=2 cases were diagnosed with NSCLC Favor squamous cell carcinoma, N=1 was diagnosed with NSCLC favor adenocarcinoma, and n=3 NSCLC NOS

Table 2: Distribution of cases following preliminary diagnosis on H&E.

Diagnosis	*Frequency*	*Percentage*
Small cell carcinoma	3	9.09
Squamous cell carcinoma	3	9.09

Adenocarcinoma	5	15.15
Non-Small Cell Lung Carcinoma in favor of Squamous Cell Carcinoma	11	33.33
Non-Small Cell Lung Carcinoma in favor Adenocarcinoma	2	6.06
Non-Small Cell Lung Carcinoma Not Otherwise Specified	8	24.24
Large Cell Neuroendocrine Tumor	1	3.03
TOTAL	33	100%

There were some variations with the diagnosis following immunohistochemistry, specifically in the diagnosis of Non-Small Cell Lung Carcinoma Not Otherwise Specified (NSCLC NOS), which is the diagnosis that requires more clarity to start treatment. The number of Non-Small Cell Lung Carcinoma Not Otherwise Specified cases reported by H & E (table 2) was 24.24% by IHC and was reduced to 9.09%. Out of the total n=5 cases the diagnosis following staining by TTF-1 and p63 all the n=5 cases were diagnosed as Non-Small Cell Lung Carcinoma in favor of Adenocarcinoma because they were found to be positive for TTF-1 and negative for p63. To summarize the most important result from the IHC was the diagnoses that were derived from the previously grouped NSCLC NOS. There was a 15.15% reduction of cases from this group with n= cases getting a clearer diagnosis. The remaining n=2 cases were found to be negative for both TTF-1 and p63 and could not be subclassified as depicted in table 3.

Table 3: Distribution of cases following diagnosis by IHC.

Diagnosis	*Frequency*	*Percentage*
Small cell carcinoma	3	9.09
Squamous cell carcinoma	2	6.06
Adenocarcinoma	5	15.15
Non-Small Cell Lung Carcinoma in favor of Squamous Cell Carcinoma	13	39.39
Non-Small Cell Lung Carcinoma in favor Adenocarcinoma	7	21.21
Non-Small Cell Lung Carcinoma Not Otherwise Specified	3	9.09
TOTAL	33	100%

One of the important observations of the study was that the markers TTF-1 and p63 are mutually exclusive in the data studied. When one has taken up the stain the other has not. It has happened that both have been negative but both being positive in the same case has not been found in the data that has been analyzed in the n=33 cases studied. (Table 4)

Table 4: Diagnosis of H&E and expression of TTF-1 and p63

Diagnosis	*Frequency*	*Positive for TTF-1*	*Negative for TTF-1*	*Positive for p63*	*Negative for p63*
Small cell carcinoma	3	2	1	0	3
Squamous cell carcinoma	3	0	3	3	0
Adenocarcinoma	5	5	0	0	5

Non-Small Cell Lung Carcinoma in favor of Squamous Cell Carcinoma	11	1	10	9	2
Non-Small Cell Lung Carcinoma in favor Adenocarcinoma	2	2	0	0	2
Non-Small Cell Lung Carcinoma Not Otherwise Specified	8	5	3	3	5
Large Cell Neuroendocrine Tumor	1	1	0	0	1
TOTAL	33	16	17	15	18

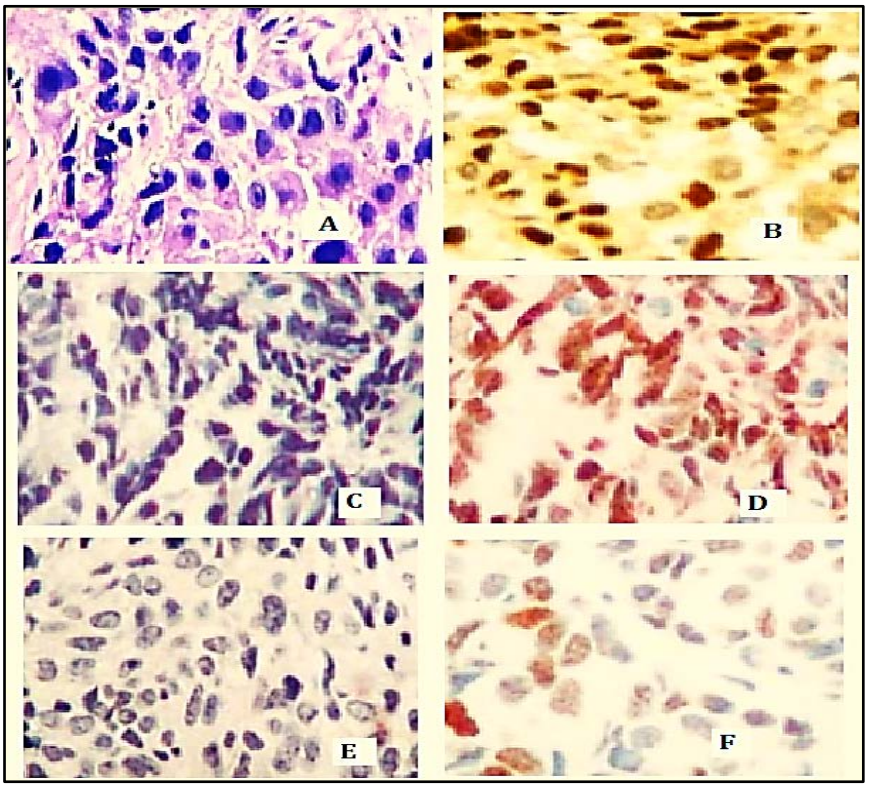

Figure 1: A: Non-small cell lung carcinoma in favor of squamous cell carcinoma (H & E); B: p63 immunostaining of the lesion in A; C: Small Lung Carcinoma thyroid transcription factor–1 TTF-1 immunostaining; D: Same sample of case C with Carcinoma thyroid transcription factor–1 TTF-1 immunostaining; E: Poorly differentiated squamous cell carcinoma tissue sample p63 immunostaining; F: same Specimen as E with thyroid transcription factor–1 TTF-1 immunostaining;

Discussion

In the current study, the highest frequency of lung carcinomas occurred in the age group of 51 – 60 years with 36.36% of cases, followed by the age group 61 - 70 with 25% of cases. Worldwide most lung cancers are diagnosed in the 6th and 7th decades of life. [8] In this study, the number of cases for males was 75.75% and for females, it was 24.24%. The lung cancer distribution across the world is 68% in males and 32% in females. [1] but in developing nations such as India, the percentage of males is higher than females. This is because the percentage of women smoking is less in developing nations. In this study, all the NSCLC cases (If we

consider everything except small cell carcinoma cases and the LCNEC case) account for 29 out of the 33 cases. That is a percentage of 87.88%, this is following the worldwide average of 80% of all the lung carcinoma cases being NSCLC cases. [9] The immunohistochemical research of lung biopsies is a fascinating and varied endeavor; in this work, two markers, TTF-1 and p63, were chosen, both of which have a wealth of data derived from previous investigations. On a preliminary H&E report, p63 stained 87 percent of the cases suspected of being squamous cell carcinoma, and TTF-1 stained all of the n=5 cases suspected of being adenocarcinoma in this study. Because there were n=5 instances suspected to be adenocarcinomas on H&E, this 100% staining could be attributed to the small number of cases. There was a 15.15% reduction of cases from this group with n= cases getting a clearer diagnosis. The remaining n=2 cases were found to be negative for both TTF-1 and p63 and could not be subclassified. Immunohistochemistry analysis of TTF-1 and p63 expression has demonstrated to be useful in distinguishing between small-cell lung carcinomas and poorly differentiated pulmonary squamous cell carcinomas. TTF-1 and p63 positivity rates in small-cell lung carcinomas and poorly differentiated pulmonary squamous cell carcinomas were consistently high in previous research on tissue or cell block sections. Negative staining is sometimes as important as a positive stain. If a stain falls on either side of the fence it can be very useful for diagnostic purposes, this is the case with the p63 stains relationship with adenocarcinoma. In this thesis there were n=5 cases of adenocarcinoma diagnosed on H&E, this includes the groups diagnosed as adenocarcinoma and NSCLC favor adenocarcinoma but this is excluding the cases from the group of NSCLC NOS that were later classified as NSCLC favor adenocarcinoma based on IHC. Out of the n=5 cases, none (0%) of them were positive for p63. The Marker p63 is seen to be consistently negative in many of the studies conducted. [10] TTF-1 is expressed in 90–100% of small-cell lung carcinomas and is almost undetectable in pulmonary squamous cell carcinomas with weak differentiation. [11-14] Small-cell lung carcinomas do not express p63, while 90–100% of poorly differentiated pulmonary squamous cell carcinomas are immunoreactive with p63 antibodies. [15-16] The TTF-1 +/p63 phenotype is present in 87–94% of small-cell lung carcinomas, while the p63 +/TTF-1 phenotype is present in 96–100% of poorly differentiated pulmonary squamous cell carcinomas, according to tissue and cell block sections stained with TTF-1 and p63 antibodies. [17-19] A wide range of tests are often required to arrive at a definitive diagnosis, but because the tissue in a biopsy is so restricted, we must limit ourselves to the procedures that provide good results. [20]

Conclusion

The results of this study within the limitations show that TTF-1 and P63 can be considered reliable indicators for further sub-tying of non-small cell lung cancer. TTF-1 and P63 IHC are useful for distinguishing between adenocarcinoma and squamous cell carcinoma, which is significant given that novel medicines have been developed with varying therapeutic effects depending on the histologic type. As a result, adding immunohistochemistry for identifying non-small cell lung carcinomas should be considered in all the cases.

References

1. Sung, H, Ferlay, J, Siegel, RL, Laversanne, M, Soerjomataram, I, Jemal, A, Bray, F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021: 71: 209 - 249.
2. Ordonez NG. Thyroid transcription factor-1 is a marker of lung and thyroid

carcinomas. Adv Anat Pathol 2000;7(2):123-27.
3. Ordonez NG. Utilization of thyroid transcription factor-1 immunostaining in the diagnosis of lung tumors. Methods Mol Med 2003; 75:355-68.
4. Shih Ie M, Kurman RJ. P63 expression is useful in the distinction of epithelioid trophoblastic tumors by profiling trophoblastic subpopulations. Am J Surg Pathol 2004;28(9):1177-83.
5. Philip T Cagle, Lucian R Chirieac. Advances in treatment of lung cancer with targeted therapy: Arch Pathol Lab Med,2012;136:504.
6. David C Hoak. Distinguishing between adenocarcinoma and squamous cell carcinoma of the lung. Diagnostic Cytopathology. 2005; 33:223-227.
7. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Et al. International association for the study of lung cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma. J Thorac Oncol. 2011; 6(2):244-85.
8. Neville HL, Hogan AR, Zhuge Y, et al. Incidence and outcomes of malignant pediatric lung neoplasms. J Surg res 2009; 156 (2): 224-30.
9. Devesa SS, Bray F, Vizcaino AP, Parkin DM. International lung cancer trends by histologic type. Int J Cancer 2005;117(2):294-99.
10. Shtilbans V, Szporn AH, Wu M, Burstein DE. P63 immunostaining in the stained bronchoscopic cytological specimens. Diagn Cytopathol 2005; 32 (4): 198-203.
11. Kaufmann O, Dietel M. Thyroid transcription factor-1 is the superior immunohistochemical marker for pulmonary adenocarcinomas and large cell carcinomas compared to surfactant proteins A and B. Histopathology 2000;36:8–16.
12. Zamecnik J, Kodet R. Value of thyroid transcription factor-1 and surfactant apoprotein A in the differential diagnosis of pulmonary carcinomas: a study of 109 cases. Virchows Arch 2002; 440:353–361.
13. Folpe AL, Gown AM, Lamps LW, et al. Thyroid transcription factor-1: immunohistochemical evaluation in pulmonary neuroendocrine tumors. Mod Pathol 1999; 12:5–8.
14. Byrd-Gloster AL, Khoor A, Glass LF, et al. Differential expression of thyroid transcription factor 1 in small cell lung carcinoma and Merkel cell tumor. Hum Pathol 2000; 31:58–62.
15. Kaufmann O, Fietze E, Mengs J, et al. Value of p63 and cytokeratin 5/6 as immunohistochemical markers for the differential diagnosis of poorly differentiated and undifferentiated carcinomas. Anatomic Pathol 2001; 116:823–830.
16. Wang BY, Gil J, Kaufman D, et al. p63 in pulmonary epithelium, pulmonary squamous neoplasms, and other pulmonary tumors. Hum Pathol 2002; 33:921–926.
17. Wu M, Wang B, Gil J, et al. p63 and TTF-1 immunostaining. A useful marker panel for distinguishing small cell carcinoma of lung from poorly differentiated squamous cell carcinoma of lung. Am J Clin Pathol 2003; 119:696–702.
18. Zhang H, Liu J, Cagle PT, et al. Distinction of pulmonary small cell carcinoma from poorly differentiated squamous cell carcinoma: an immunohistochemical approach. Mod Pathol 2005; 18:111–118.
19. Wu M, Szporn AH, Zhang D, et al. Cytology applications of p63 and TTF-1 immunostaining in the differential diagnosis of lung cancers. Diagn Cytopathol 2005;33: 223–227.
20. Manfred, D. May There Exist Healthy Diseases?. Journal of Medical Research and Health Sciences 2002; 5(3), 1801–1803. https://doi.org/10.52845/J MRHS/2022-5-3-1