International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2023; 6(3): 24-28
Received: 21-04-2023
Accepted: 01-06-2023

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Role of Napsin-A and TTF-1 in differentiating primary from secondary lung carcinoma

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DOI: https://doi.org/10.33545/pathol.2023.v6.i3a.531

Abstract
Background: So far lung carcinoma is the 2nd most prevalent cancer in both male and female and remains one of the leading cause of death in the world. With recent advancements in molecular testing and Immunohistochemistry (IHC), it has been possible to subtype various lung carcinomas. The combination of histopathological classification and Immunohistochemistry provides clinical insight into the management of these patient. Napsin-A and TTF-1 are two IHC markers with the potential to help us differentiate primary from metastatic lung carcinoma.
Materials and Methods: The present study was carried out on 108 patients. Types of samples include lung biopsy specimens received in histopathology section of the pathology department. The cases were diagnosed based on light microscopy and immunohistochemistry procedure with Napsin-A and TTF-1 antibodies was performed.
Aims and Objectives
- To determine the incidence of primary lung adenocarcinoma as compared to other lung malignancy.
- To use Immunohistochemistry markers, Napsin-A and TTF-1, for differentiating primary lung malignancy from metastatic lung cancer.

Results: A total of 108 lymph node specimens were studied. Age distribution varied from 35-82 years with male preponderance. Out of 65 cases of adenocarcinoma of lung, 53 (81.5%) expressed both Napsin-A and TTF-1 positivity, 07 (10.7%) were Napsin-A positive but TTF-1 negative while 05 (07.6%) were negative for both the markers. Out of 03 cases of adenosquamous carcinoma, 01 (33.3%) was Napsin-A positive, TTF-1 negative while 02 (66.7%) were negative for both. There were 06 cases of squamous cell carcinoma, 01 (16.7%) showed TTF-1 positivity but negative for Napsin A, while 05 (83.3%) expressed none of the markers. Out of 25 cases of secondaries to lung, 12 (48%) expressed TTF-1 positivity only while 13 (52%) expressed none of the two markers.
Conclusion: Performing an IHC with dual markers Napsin A and TTF-1 increases the sensitivity of detecting primary lung malignancy.

Keywords: Lung carcinoma, metastatic lung cancer, Napsin-A, TTF-1, immunohistochemistry

Introduction

Differentiation of primary lung adenocarcinoma from metastasis can be challenging as detecting origin of those metastasis from unknown primary can be perplexing. Hence it demands sensitive and specific biomarkers to narrow down the differential diagnosis and come to an accurate conclusion. So far lung carcinoma are the 2nd most prevalent cancer in both male and female and remains one of the leading cause of death in the world [1, 2]. TTF-1 (Thyroid Transcription Factor-1) and Napsin-A are two of the most commonly used bio markers for diagnosing lung malignancy. Our present study aims to determine the role of Napsin-A and TTF-1 in differentiating a primary lung Ca from the metastasis.

Napsin-A is one of the recently discovered biomarker used to detect primary lung adenocarcinoma [3, 4] as it gives granular cytoplasmic positivity in malignant cells in patients with adenocarcinoma of lungs upon performing IHC procedure. It's a type of functional aspartic proteinase and is normally expressed in lung parenchyma in type II pneumocytes. Its involved in maturation of biologically active surfactant SP-B [5, 6, 7]. On the other hand TTF-1 is a homeodomain-containing nuclear transcription factor which is expressed in forebrain, thyroid and lung, making it a widely used reliable marker for detection of adenocarcinoma of lung [8]. It gives nuclear positivity in IHC study.

Immunohistochemical expression of Napsin A and TTF-1 was studied in 108 patients with lung carcinoma including 25 metastatic carcinoma. Napsin-A was expressed in 62/108 cases of lung carcinoma while no expression was noted in any of the 25 metastatic carcinoma. In contrast, TTF-1 was positive in 69/108 cases of lung carcinoma including 12/25 cases with metastasis. 16/108 cases were such that they showed neither TTF-1 nor Napsin-A positivity. Furthermore, the sensitivity and specificity expression was associated with gender, smoking history, performance status, pathological type, primary tumor size and nodal metastasis.

Lung carcinoma is one of the most frequently encountered carcinoma in clinical setting accounting for approximately 55.9% & 36.6% deaths per 1,00,000 male and female respectively making it the most common cause of cancer related death. The most common malignant finding among lung cancer is primary lung adenocarcinoma [9, 10]. Its occurrence is still on the rise every year. Ensuring accurate identification & classification is of paramount importance as it'll provide clinical insight into management of the patient. The combination of histological pattern and immunohistochemistry plays an important role here. Compared with other techniques, immunohistochemistry has a number of advantages, including being widely available, technically less challenging, and cost-efficient with a rapid turn-around time. Thus, molecular-specific immunohistochemical assays have huge potential as practical screening tools for the detection of druggable genetic alterations and for the assessment of biomarkers for molecular-targeted therapy [11]. Due to its abundant blood supply, lung is the most common site for metastasis from tumor arising from other sites in the body. TTF-1 is primarily used to distinguish adenocarcinomas of lung (and thyroid) origin from carcinomas of other sites and to distinguish lung adenocarcinomas and small cell lung cancers from squamous cell carcinomas [25]. While Napsin-A is expressed in defining primary adenocarcinoma of the lung.

The present study aims at analyzing the outcome of combined use of Napsin A and TTF-1 is distinguishing primary from metastatic lung carcinoma.

Methods and Material

Patient selection and tissue sample collection

All patients with lung cancer in this study were selected from Shri M. P. Shah Government Medical College, Hospital Jamnagar, Gujarat, during the period of August 2018- August 2022. The pathological tissue specimen and clinical data for each patient were collected prior to treatment. The clinical data included name, age, gender, smoking history, histopathological diagnosis, grade of tumor differentiation, tumor stage, primary tumor size and nodal metastasis. Histological sub classification was carried out according to the World Health Organization (WHO) classification. In total, 83 patients with primary lung cancer and 25 patients with metastatic lung cancer met our study criteria.

Among 108 patients, 92 were male patients and 16 were female patients. The gender ratio (5:1).

Table 1: Age wise occurrence of lung carcinoma

Age group	No. of patients
<50	21
51-64	34
65-74	45
75-85	06
>85	02

The median age range was 65-74 years. A total of 45 patients had the history of smoking and 96 patients had symptoms when first diagnosed, such as fever, cough with expectorants, hemoptysis, chest pain, weakness and breathlessness. Adenocarcinoma (85%) was the most common tumor type in the patients followed by squamous cell (06%) small cell carcinoma of lung (04%), Adenosquamous cell carcinoma (03%), large cell carcinoma (02%). A total of 71 patients (66%) had stage I-II disease and the remaining 37 (34%) had stage III-IV disease. There were 65 patients with nodal metastasis and 59 patients with a primary tumor size > 3 cm when diagnosed.

Of the 25 cases with metastatic lung cancer, 09 cases developed from colon carcinoma, 07 cases from thyroid carcinoma, 05 developed from uterine cercix cancer and 04 cases developed from mammary adenocarcinoma.

Immunohistochemistry

All specimens were routinely fixed in 10% buffered neutral formalin and embedded in paraffin. Each section (5.0 µm) was stained with the standard hematoxylin and eosin method for screening.

IHC was performed using an EnVision two step method. Primary antibody included anti-napsin A and anti-TTF-1 antibody (Ready to Use BioGenex, mouse monoclonal Fremont). The paraffin was removed from the slides by Xylene, and the tissue was rehydrated in various concentrations of ethanol. Hydrogen peroxide (3%) was added to eliminate endogenous peroxidase activity. The slides were then processed using steam heat retrival for 30 min. The antibody was incubated for 60 min at room temperature. The above steps were performed using automatically in Dako automatic immunostainer. Negative controls for napsin A and TTF-1 were carried out by omitting the primary antibody. The positive controls were previously known napsin A or TTF-1 positive lung tissues.

Scoring

For Napsin A, only a granular cytoplasmic staining pattern was accepted as positive, while for TTF-1, only a nuclear staining pattern was considered to be positive. The stained slides were observed microscopically by two experienced pathologists.

Results

Napsin-A positivity was interpreted as granular cytoplasmic staining. Internal control for which was normal lung tissue type-II pneumocytes. Napsin-A was expressed in 60/65 (92.0%) cases of adenocarcinoma of lung, while 02/03 cases (66.0%) of adenosquamous carcinoma of lung showed Napsin-A positivity but was negative in other types of primary lung cancer and metastatic lung cancer cases.

Table 2: Positive expression of napsin A and TTF-1 in lung cancer

Histology	Napsin A	TTF-1
PLC	62/83 (74.6%)	57/83 (68.7%)
AdC	60/65 (92.0%)	53/65 (81.5%)
SCC	00/06 (0.0%)	01/06 (16.7%)
SCLC	00/05 (0.0%)	02/05 (40.0%)
ASCC	02/03 (66.0%)	01/03 (33.0%)
LCC	00/04 (0.0%)	00/04 (0.0%)
MLC	00/25 (0.0%)	12/25 (48.0%)

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PLC, primary lung cancer; MLC, metastatic lung cancer; AdC, adenocarcinoma; SCC, squamous cell carcinoma; SCLC, small-cell lung carcinoma; ASCC, adenosquamous cell carcinoma; LCC, large- cell carcinoma.

TTF-1 was expressed in Adenocarcinoma of lung 53/65 cases (81.5%) and few cases of squamous cell lung cancer and adenosquamous lung cancer. It was positive in 12/25 (48.0%) metastatic lung carcinoma making it a favorable marker to detect the secondaries to the lung from organs like colon, thyroid etc. It gave a nuclear positivity without any membraneous or cytoplasmic positivity. Internal control for TTF-1 was taken as nuclear staining of some normal pulmonary alveolar pneumocytes near the lesion.

Table 3: Napsin A and TTF-1 expression in primary lung carcinoma v/s metastatic lung carcinoma.

	$Napsin^{+}/TTF^{+}$	$Napsin^{+}/TTF^{-}$	$Napsin^{-}/TTF^{+}$	$Napsin^{-}/TTF^{-}$
Adenocarcinoma	53 (81.5%)	07 (10.7%)	00	05 (07.6)
Adenosquamous Carcinoma	01	02(66.7%)	00	01 (33.3%)
Squamous cell carcinoma	00	00	01 (16.7%)	05 (83.3%)
Small cell carcinoma	00	00	02 (40%)	03 (60%)
Metastasis to lungs	00	00	12 (48.0%)	13 (52.0%)

Combining napsin A and TTF-1 resulted in sensitivity to lung adenocarcinoma increasing to 81.5% (53/65), whereas for confirming that the lesion is secondary from other organ and not a primary lung malignancy (Napsin-1 negative and TTF-1 positive) sensitivity was 48.0%.

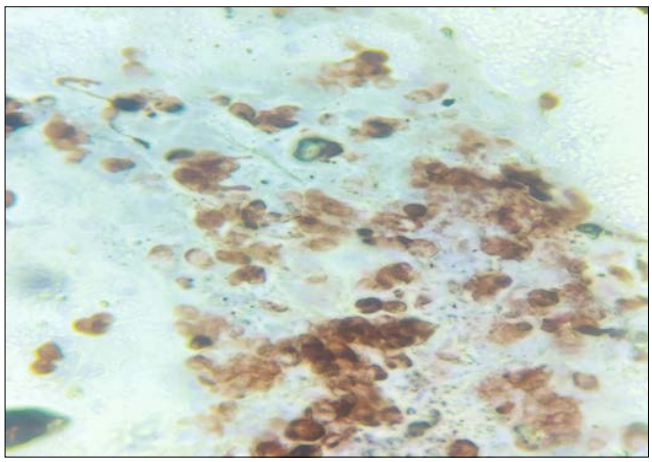

Fig 1: Napsin A is expressed in the cytoplasm of primary lung adenocarcinoma with a granular staining pattern (EnVision two-step method) (magnification: ×200)

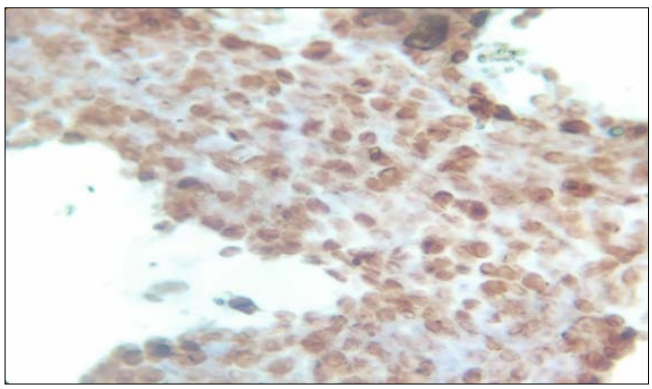

Fig 2: TTF-1 is expressed in the nucleus of primary lung adenocarcinoma (EnVision two-step method) (magnification: ×200)

Discussion

Lung carcinoma is the leading cause of cancer related mortality regardless of the gender. It is categorized into two main groups: small cell lung carcinoma (SCLC, 15% of all lung cancers) and Non-small cell lung carcinoma (NSCLC) that accounts for approximately 80% to 85% of all lung cancers. It's heterogeneous in nature, comprising many histological sub-types. For example, adenocarcinoma (ADC) accounts for approximately 50% to 70% and squamous cell carcinoma (SqCC) for approximately 20% to 30% of all NSCLC cases [12-13]. Because the treatment and prognosis of patient with lung carcinoma varies with grading and histological subclassification of the tumor, it is essential to detect the subtype accurately. It is equally important to find out the origin of primary tumor in case of metastasis to lungs.

Novel aspartic proteinase of the pepsin family A (Napsin A, TAO1/TAO2) belongs to the peptidase A1 family, such as Cathepsin E, renin, and pepsin and is encoded by the *NAPSA* gene located at chromosome 19q13.3 [14-16]. Napsin A is mainly expressed in the cytoplasm of type II pneumocytes, intra-alveolar macrophages, proximal and convoluted renal tubules, and pancreatic acini and ducts [17, 18] as well as adenocarcinomas of the lung, papillary renal cell carcinomas, and ovarian clear cell carcinomas. The expression of napsin A was significantly higher in patients without nodal metastasis or symptoms, or with a primary tumor size <3 cm, compared with their counterparts. It was also found to be associated with a high degree of differentiation in adenocarcinoma in accordance with the study done by Peng Zhang *et al.* [26]. These findings indicate napsin A may be important in the carcinogenesis of lung cancer. Hense to differentiate adenocarcinoma of the lung from pulmonary metastases of extrapulmonary origin and to support a pulmonary origin of metastases from unknown primary tumors Napsin A IHC has proved to be of utmost importance. Napsin A was re-expressed in the tumorigenic HEK 293 kidney cell line. Cells expressing napsin A showed a reduced capacity for anchorage-independent growth, and formed tumors in SCID mice with a lower efficiency and slower onset compared to vector-transfected control cells. On the other hand, napsin A staining was associated with tumor differentiation. Taken together, these observations suggest the crucial role napsin A plays in lung cancer. The loss of napsin A expression may enhance the

aggressive behavior of lung cancer, influencing disease prognosis [19].

Apart from the TNM staging, TTF-1 was identified as an independent prognostic factor for survival [20]. Expression of TTF-1 is associated with better prognosis if detected earlier in the course of the lesion in cases of non small cell carcinoma of lung as proved by meta-analysis by Berghmans *et al.* [21].

In our current study of 83 primary lung cancer samples, napsin A and TTF-1 had a very similar sensitivity. Although TTF-1 was expressed in 81.5% of the adenocarcinoma specimens, its expression was also noted in small-cell lung carcinomas, as well as some of the squamous cell carcinomas, in accordance with previous studies done by Tan D *et al.* and Kaufmann O *et al.* [22, 23]. Furthermore, by virtue of its tissue-specific exclusive expression in thyroid neoplasms, it would be difficult to distinguish primary lung adenocarcinoma from metastatic lung adenocarcinoma from the thyroid based on clinical and morphological features. In contrast, napsin A is much more specific to lung adenocarcinoma. Although certain renal cell carcinomas have been reported to express napsin A, it is thought that these false positives are likely due to the presence of intrinsic biotin, which is readily detected on negative controls [24].

Our study concludes that even though chances of false negative using Napsin-A and TTF-1 may exist in case of adenocarcinoma of lung, they can be substantially reduced on using these 2 markers together and TTF-1 positivity with Napsin-A negativity guides the diagnosis more towards the metastatic lung carcinoma. In a clinical set-up, the combined use of napsin A and TTF-1, as well as morphology and clinical information, can facilitate the diagnosis and differentiation of lung cancer.

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How to Cite This Article
Gojiya J, Aghera G, Atara A, Dethariya K. Role of Napsin-A and TTF-1 in differentiating primary from secondary lung carcinoma. International Journal of Clinical and Diagnostic Pathology. 2023;6(3):24-28.