Endocrine Pathology (2020) 31:39–45
https://doi.org/10.1007/s12022-019-09600-6

Napsin A Expression in Subtypes of Thyroid Tumors: Comparison with Lung Adenocarcinomas

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Published online: 1 December 2019

Abstract
Napsin A is widely used in the diagnosis of lung adenocarcinoma and has also been reported to be positive in cases of thyroid carcinomas. We investigated napsin A levels through immunohistochemistry on whole sections of 210 primary thyroid tumors of various subtypes and another 41 metastatic thyroid carcinomas, and compared these with 125 primary and 25 metastatic lung adenocarcinomas. The results showed that napsin A was expressed in 23.8% thyroid tumors and 30.3% papillary thyroid carcinomas. Most cases showed a focal and weak to moderate expression. In comparison, 80.8% primary lung adenocarcinomas expressed napsin A, with mostly diffused and strong expression. For metastatic carcinomas of thyroid and lung origin, napsin A was detected in 39.0% of thyroid carcinomas in contrast to 88.0% in cases of lung adenocarcinomas. Comparisons of additional markers, TTF-1, CK7, thyroglobulin, and Pax-8 in metastatic carcinomas showed the overlapping expression of immunomarkers of TTF-1 and CK7. Thyroglobulin and Pax-8 were useful for distinguishing between metastatic carcinomas; however, Pax-8 may be a superior marker due to its higher sensitivity. The clinicopathological analysis of papillary thyroid carcinomas showed that the expression of napsin A was positively correlated with lymph node metastasis ($p = 0.030$). Here, we focused on the expression of napsin A in thyroid tumors and compared it with that in lung adenocarcinomas. The expression of napsin A is common in thyroid tumors and the combined expression of napsin A and TTF-1 in a metastatic thyroid carcinoma is a cause for concern due to chances of misdiagnosis as lung adenocarcinoma.

Keywords Napsin A · Thyroid tumor · Lung adenocarcinoma · Lymph node metastasis

Introduction

Napsin A is an aspartic proteinase that has been widely applied as a useful marker for diagnosis of lung adenocarcinoma recently. Immunohistochemistry (IHC) for napsin A and TTF-1 was recommended in the 2015 World Health Organization classification of lung tumors in typing of non-small cell carcinomas [1]. Previous studies suggest that the combined immunomarkers of napsin A and TTF-1 were also specific for primary lung adenocarcinoma and helpful in segregating it from non-pulmonary carcinoma [2–5]. However, other studies showed that napsin A was positive in a series of carcinomas arising from other sites including renal, thyroid, colon, prostate, bile duct, pancreas, breast liver, and esophagus as well as in clear cell carcinomas of the female genital tract [6–10]. Among these non-pulmonary tumors, napsin A expressing thyroid tumors which are mostly TTF-1 positive are significant as they may metastasize to the lung and mimic lung adenocarcinomas in some cases.

It has been reported that thyroid carcinomas are positive for napsin A with variable results as it is seen in rare cases and sometimes in approximately half the cases. In previous studies, using tissue microarrays, Bishop et al. positively detected napsin A in 2 of 38 cases (5%) of tall cell variant of papillary

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thyroid carcinoma [6]. Kim et al. found that napsin A was expressed in only 3 of 98 cases (3.1%) with lower expression scores in metastatic thyroid tumor in contrast to 47 of 54 cases (87%) of metastatic pulmonary adenocarcinomas [5]. However, other studies stated the limitations of napsin A in distinguishing lung adenocarcinomas from carcinomas of other sites including thyroid carcinomas. Kadivar et al. found that napsin A was positive in 14 of 30 cases of thyroid tumor and concluded that napsin expression might lead to misdiagnosis of metastatic thyroid carcinoma as lung adenocarcinoma [7]. Mukhopadhyay et al. detected napsin A expression in 5 of 10 cases (50%) of metastatic papillary thyroid carcinomas [8]. More importantly, Chernock et al. investigated napsin A expression in high-grade and micropapillary patterns of thyroid carcinomas, and showed that napsin A was expressed in a minority of poorly differentiated and undifferentiated thyroid carcinomas, especially in the cases with a micropapillary pattern [11]. Different findings from a small number of cases in these studies may lead to confusion regarding the application of napsin A in the differentiation of carcinomas as that of thyroid or lung origin. Thus, the evaluation of napsin A expression in a relatively large number of thyroid tumors and comparison to lung adenocarcinoma is required. Moreover, it was noticed that the frequency of napsin A expression may be higher in lymph node metastasis of papillary thyroid carcinomas in the observations of Mukhopadhyay et al. and Chernock et al. [8, 11]. However, data on napsin A expression of thyroid carcinomas and its correlation with clinicopathologic parameters has not been investigated.

This study aimed to evaluate the prevalence of napsin A expression in various subtypes of thyroid tumors focusing on the comparison with lung adenocarcinomas and to explore its correlation with clinicopathologic parameters. We also compared a panel of commonly used immunomarkers such as CK7, thyroglobulin (Tg), and Pax-8 in the diagnosis of metastatic carcinomas. The differential diagnosis of metastatic carcinomas of thyroid and lung origin will be particularly discussed in this study.

Methods

Case Selections

The cases included 210 primary thyroid tumors, 125 primary lung adenocarcinomas, 41 metastatic thyroid carcinomas, and 25 metastatic lung adenocarcinomas. Primary thyroid tumors included 155 papillary thyroid carcinomas (PTC), 16 poorly differentiated thyroid carcinomas (PDTC), 9 anaplastic thyroid carcinomas (ATC), 1 Hürthle cell carcinoma, 6 medullary thyroid carcinomas (MTC), 12 follicular thyroid carcinomas (FTC), and 11 follicular thyroid adenomas (FTA). One hundred fifty-five cases of PTC were analyzed for clinicopathologic parameters including age, sex, tumor size, TNM stage, extrathyroidal invasion, and lymph nodal metastasis. All cases of PTC in this study were performed with cervical lymph node dissection and 30 patients were detected with lymph nodal metastasis by pathological examination. Among these corresponding lymph nodal metastases of PTC, twenty-one metastases which were greater than 0.5 cm in size were tested for napsin A via staining for comparison with that in primary carcinoma. Primary lung adenocarcinomas were generally classified as minimally invasive, lepidic, acinar, papillary, micropapillary, mucinous, enteric, or solid variant according to their predominant histologic patterns. A total of 66 metastatic carcinomas were analyzed including 41 metastatic carcinomas of thyroid origin and 25 metastatic carcinomas of lung origin. They were confirmed on the basis of previous biopsy specimens or resections of known primary carcinomas or a clinical history of carcinoma. Metastatic carcinomas of thyroid origin consisted of 37 cases of PTC, 2 cases of FTC, and 2 cases of PDTC. This study was approved by the Ethical Review Committee of Tianjin Medical University Cancer Institute and Hospital.

Immunohistochemistry

Immunohistochemical staining was performed on paraffin-embedded tissue sections using an automated Ventana BenchMark XT system (Roche, Ventana Medical Systems, Inc., Tucson) with appropriate positive and negative controls. IHC was carried out on the corresponding unstained sections from each case using commercially available antibodies from Maxim bio-technologies Co., Ltd. (Fuzhou, China), as follows: napsin A (monoclonal, mouse, MX015, prediluted), TTF-1 (monoclonal, MX011, mouse, prediluted), CK7 (monoclonal OV-TL 12/30, mouse, prediluted), Thyroglobulin (monoclonal, TGB04+TGB05, mouse, prediluted), and Pax-8 (Polyclonal, rabbit, prediluted).

The immunostained slides of napsin A were scored by two co-authors (J W and Y Z). Coarse granular cytoplasmic staining of tumor cells was considered positive for napsin A, and the staining extent was classified as follows: < 1% = negative; score 1 = 1 to 25% of tumor cells positive; score 2 = > 25 to 50%; score 3 = > 50%. Staining intensity was characterized as weak (score 1), moderate (score 2), or strong (score 3). The staining score and percent positive cells were added to obtain a total immunostaining score, and a score ≥ 2 was considered as positive. In this study, score 2 was considered as low expression, score 3–4 was considered as moderate expression, and score 5–6 was considered as high expression. As in case of other antibodies, nuclear staining for TTF-1 and Pax-8 and cytoplasmic and/or membrane staining for CK7 and Tg were observed. The presence of more than 1% expression of these markers within tumor cells was considered positive.

Statistical Analysis

Data were analyzed using the SPSS statistical package (version 22.0; SPSS, Inc., Chicago, IL). Crosstabs, Pearson's χ^2 tests, and Fisher's exact test were used as needed. A value of $p < 0.05$ was regarded as significant.

Results

The Results of IHC Staining for Napsin A in Subtypes of Thyroid Tumors and Lung Adenocarcinomas

Of the 210 cases of thyroid tumors, napsin A was positive in 50 cases (23.8%), with low expression in 24 cases (11.4%), moderate expression in 20 cases (9.5%) and high expression in 6 cases (2.9%). Napsin A was observed in 47 of 155 cases (30.3%) of PTC with low expression in 23 cases (14.8%), moderate expression in 18 cases (11.6%), and high expression in 6 cases (3.9%). Napsin A was detected in 2 of 16 cases (12.5%) of PDTC with moderate expression and 1 of 11 cases (11.1%) of ATC with low expression. Napsin A expression was not observed in one case of Hürthle cell carcinoma, 6 cases of MTC, 12 cases of FTC, and 11 cases of FTA. Of the 155 cases of PTC, napsin A was positive in 25 of the 64 cases (39.1%) of classic PTC, 12 of the 46 cases (26.1%) of papillary microcarcinoma variant (PTMC), 3 of the 5 cases (60%) of diffuse sclerosing variant (DSVPTC), 3 of the 10 cases (30.0%) of Tall cell variant, and 4 of the 8 cases (50.0%) of solid/trabecular variant of PTC. The positive expression of napsin A was in 12 of 21 (57.1%) in corresponding Cervical lymph node metastasis of PTC, higher than general papillary thyroid carcinoma (57.1% vs. 30.3%, $p = 0.015$).

Of 125 cases of lung adenocarcinoma, napsin A was observed in 101 of 125 cases (80.8%) including low expression in 2 of 101 cases (1.98%), moderate expression in 19 of 101 cases (18.8%), and high expression in 80 of 101 cases (79.2%). Napsin A was positive in 4 of 4 cases (100%) of minimally invasive adenocarcinoma (MIA), 15 of 16 cases (93.8%) of lepidic subtype, 32 of 34 cases (94.1%) of acinar subtype, 16 of 18 cases (88.9%) of papillary subtype, 13 of 13 cases (100%) of micropapillary subtype, 16 of 29 cases (55.2%) of solid subtype, 3 of 8 cases (37.5%) of mucinous variant, and 2 of 3 cases (66.7%) of enteric variant.

The IHC results in thyroid tumors are summarized in Table 1. The extent and the salient staining of thyroid tumors are illustrated in Figs. 1 and 2, respectively.

Comparison of the Panel of IHC Staining in Metastatic Carcinomas of Thyroid and Pulmonary Origin

Carcinomas of thyroid origin were metastatic to cervical lymph nodes ($n = 23$), lung ($n = 7$), mediastinum lymph nodes ($n = 4$), bone ($n = 2$), brain ($n = 1$), and other sites ($n = 4$). Of

Table 1 Expression of napsin A in various variants of thyroid tumors

Thyroid tumor		Napsin A			
Expression Histologic type	*n*	Positive *n* (%)	Low (score 2)	Moderate (score 3–4)	High (score 5–6)
Total cases	210	50 (23.8)	24 (11.4)	20 (9.5)	6 (2.9)
Papillary thyroid carcinoma (PTC)	155	47 (30.3)	23 (14.8)	18 (11.6)	6 (3.9)
Classic variant	64	25 (39.1)	15 (23.4)	6 (9.4)	4 (6.3)
Papillary microcarcinoma variant	46	12 (26.1)	7 (15.2)	4 (8.7)	1 (2.2)
Follicular variant	20	0	0	0	0
Diffuse sclerosing variant	5	3 (60.0)	0	3 (60)	0
Tall cell variant	10	3 (30)	0	2 (20)	1 (10.0)
Columnar cell variant	2	0	0	0	0
Solid/trabecular variant	8	4 (50.0)	1 (12.5)	3 (37.5)	0
Corresponding cervical lymph node metastasis of PTC	21	12 (57.1) *	3 (14.3)	6 (28.6)	3 (14.3)
Poorly differentiated thyroid carcinoma	16	2 (12.5)	0	2 (12.5)	0
Anaplastic thyroid carcinoma	9	1 (11.1)	1 (11.1)	0	0
Hürthle cell carcinoma	1	0	0	0	0
Medullary thyroid carcinoma	6	0	0	0	0
Follicular thyroid carcinoma	12	0	0	0	0
Follicular thyroid adenoma	11	0	0	0	0

*The positive expression of napsin A in corresponding cervical lymph node metastasis of PTC is similar in corresponding primary carcinomas (57.1% vs.52.4%, $p = 0.757$), and higher than general in papillary thyroid carcinoma (57.1% vs. 30.3%, $p = 0.015$)

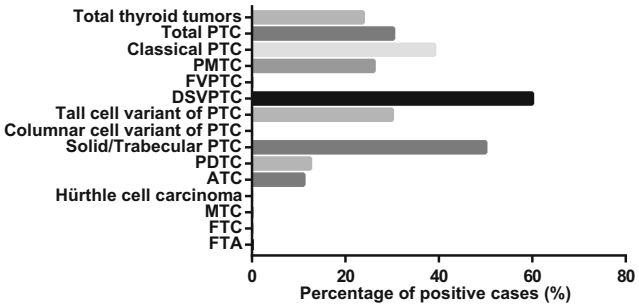

Fig. 1 The extent of napsin A positive expression in various subtypes of thyroid tumors. Abbreviations: papillary thyroid carcinomas (PTC), papillary microcarcinoma variant of PTC (PTMC), follicular variant of PTC (FVPTC), diffuse sclerosing variant (DSVPTC), poorly differentiated thyroid carcinomas (PDTC), anaplastic thyroid carcinomas (ATC), medullary thyroid carcinomas (MTC), follicular thyroid carcinomas (FTC), follicular thyroid adenomas (FTA)

41 cases, 16 cases (39.0%) were positive for napsin A staining including low expression in 9 cases (22.0%), moderate expression in 6 cases (14.6%) and high expression in one case (2.4%). Forty cases (97.6%) were positive for TTF-1. Thirty-nine cases (95.1%) were positive for CK7. Twenty-six cases (63.4%) were positive for Tg, and 39 cases (95.1%) were positive for Pax-8. The sites of metastasis in 25 metastatic lung adenocarcinomas were mediastinum lymph nodes (n = 1) and distant sites (n = 24) including cervical lymph nodes (n = 9), brain (n = 5), bone (n = 5), and other sites (n = 5). Among these cases, 22 cases (88.0%) were positive for napsin A staining including moderate expression 6 cases (24.0%) and high expression of 16 cases (64.0%). Twenty-three cases (92.0%) were positive for TTF-1. Twenty-four cases (96.0%) were positive for CK7. No cases were positive for Tg or Pax-8. The statistical analysis showed that the expression of napsin A, Tg and Pax-8 among the groups of metastatic carcinomas of thyroid and pulmonary origin was significantly different (all $p < 0.001$) considering the positive reactivity of napsin A. However, immunostaining pattern of napsin A (+)/TTF-1(+)/CK7(+) could also be seen in 14 of the 41 cases (34.1%) of metastatic thyroid carcinomas and 20 of the 25 cases (80.0%) of metastatic lung adenocarcinomas (Table 2).

The Association Between the Expression of Napsin A and Clinicopathological Characteristics in PTC

Clinicopathologic characteristics associated with napsin A expression were evaluated in 155 cases of PTC. Napsin A-positive expression was detected in 47 cases (30.3%) in PTC, and the expression of napsin A was significantly correlated with lymph node metastasis (p = 0.030). However, there was no correlation between the expression of napsin A and age (p = 0.354), sex (p = 0.084), tumor size (p = 0.395), TNM stage (p = 0.287), or extrathyroidal invasion (p = 0.189) (Table 3).

Discussion

Advances in the treatment of various cancers have increased the demand for accurate pathologic diagnosis with the assistance of immunomarkers. IHC of napsin A which was considered as a highly specific marker for lung adenocarcinoma has been reported in a variety of carcinomas of other origins. In this study, we focused on the expression of napsin A in various subtypes of thyroid tumors which are usually TTF-1 positive and that may cause confusion in the diagnosis of lung adenocarcinomas.

In our study, napsin A was expressed in 23.8% of the total 210 cases of thyroid tumors and 30.3% of PTC. Most cases (97.1%) showed low to moderate expression with a score of 2

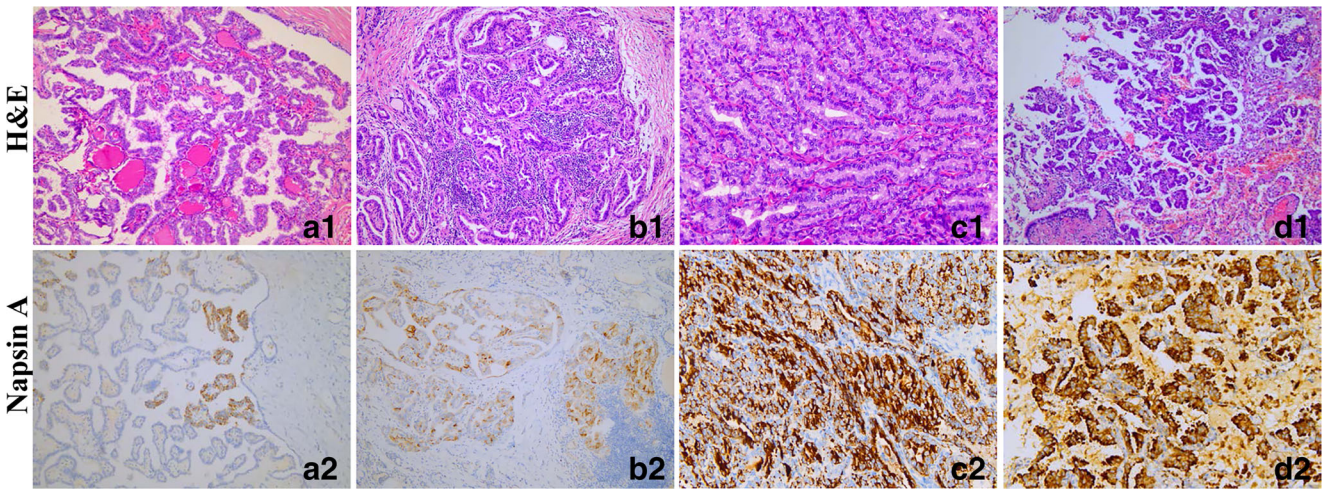

Fig. 2 Immunohistochemical staining of napsin A in thyroid tumors and lung adenocarcinoma. A1, classic variant of papillary thyroid carcinoma. A2, focal tumor cells express napsin A with moderate expression. B1, papillary microcarcinoma variant. B2, tumor cells express napsin A with weak to moderate expression. C1, tall cell variant of PTC. C2, napsin A with diffused and strong expression, such a high expression of napsin A in thyroid carcinoma is uncommon. D1, primary lung adenocarcinoma. D2, the immunostaining of napsin A is very strong and diffused

Table 2 Comparison of the metastatic sites and immunohistochemical staining in metastatic carcinomas from thyroid and pulmonary origin

Metastatic carcinomas	Thyroid origin (n = 41)	Lung origin (n = 25)	
Metastatic sites	n (%)	n (%)	
Cervical lymph node	23 (56.1)	9 (36.0)	
Lung	7 (17.1)	–	
Mediastinum	4 (9.8)	1 (4.0)	
Bone	2 (4.9)	5 (20.0)	
Brain	1 (2.4)	5 (20.0)	
Other sites	4 (9.8)	5 (20.0)	
Diagnostic markers	Positive cases (%)		p
Napsin A	16 (39.0)	22 (88.0)	< 0.001*
Weak (score 2)	9 (22.0)	0	
Moderate (score 3–4)	6 (14.6)	6 (24.0)	
Strong (score 5–6)	1 (2.4)	16 (64.0)	
TTF-1	40 (97.6)	23 (92.0)	0.552
CK7	39 (95.1)	24 (96.0)	1.000
Thyroglobulin	26 (63.4)	0	< 0.001*
Pax-8	39 (95.1)	0	< 0.001*

*A value of $p < 0.05$ was regarded as significant

to 4. Only 6 cases (2.9%) showed high expression with a score of 5 or 6 including four classic, one microcarcinoma and one tall cell variant of PTC. Similar to studies by Kadivar et al. and Mukhopadhyay et al. on whole sections, the frequency of napsin A-positive expression in thyroid tumors was more common than previous studies using tissue microarrays [7, 8]. As the expression of napsin A in thyroid tumors mostly showed focal expression it could be missed due to the limited observation of tissue microarray. Napsin A in PTC was mainly expressed in classic, microcarcinomas, diffuse sclerosing, tall cell, and solid variants of PTC. Similar to the observation of Bishop et al., napsin A seemed to be negative in a thyroid tumor which only consists of follicular structures such as follicular variants of PTC, FTC, and FTA [6]. In high-grade thyroid carcinomas, there was focal and weak staining in 2 of the 16 cases (12.5%) of PDTC and 1 of the 9 cases (11.1%) of ATC; this prevalence was lower than that reported by Chernock et al. [11]. Among primary lung adenocarcinomas, in agreement with many previous reports, 101 of 125 cases (80.8%) of lung adenocarcinoma expressed napsin A [2, 3, 12, 13]. Unlike thyroid tumors, most cases of the variants of lung adenocarcinomas showed a high expression of napsin A except for the solid and mucinous variants.

For metastatic carcinomas of thyroid and lung origin, both shared similar metastatic sites including cervical lymph node, mediastinum, bone, brain, and some other unexpected sites. The lung is a common site of thyroid carcinoma metastasis. The expression of napsin A was detected in 39.0% metastatic thyroid carcinomas with weak to moderate expression in contrast to 88% of metastatic lung adenocarcinomas with moderate to high expression. Thus, a weak or moderate expression of napsin A may not be helpful in the differential diagnosis between two carcinomas. A panel of conjunctive diagnostic

Table 3 Correlation between the expression of napsin A and clinicopathological parameters in papillary thyroid carcinomas

n = 155	Napsin A		χ^2	p
	Positive 47	Negative 108		
Age at diagnosis			0.859	0.354
≤ 45	21	57		
> 45	26	51		
Gender			2.981	0.084
Male	13	17		
Female	34	91		
Tumor size			0.724	0.395
≤ 2 cm	35	87		
> 2 cm	12	21		
TNM stage			1.134	0.287
I–II	17	49		
III–IV	30	59		
Extrathyroidal invasion			1.726	0.189
Present	31	59		
Absent	16	49		
Lymph nodal metastasis			4.703	0.030*
Present	14	16		
Absent	33	92		

The presence of the expression of napsin A (score ≥ 2) within tumor cells was considered positive

*A value of $p < 0.05$ was regarded as significant

immunomarkers was also detected in metastatic tumors. These markers are commonly used in our routine pathologic evaluation. A combination of immunomarkers of CK7, TTF-1, and napsin A is often used in the diagnosis of metastatic lung adenocarcinoma. However, regardless of the positive reactivity of napsin A expression, napsin A (+)/TTF-1(+)/CK7(+) could be observed in 14 of 41 cases (34.1%) of metastatic thyroid carcinomas. This result indicated that there was overlapping expression of these immunomarkers among the carcinomas of thyroid and lung origin. In such cases, Tg and Pax-8 were considered to be useful markers. Tg is especially expressed in tissues of thyroid origin and Pax-8 is positive in the tissues of the kidney, müllerian organs, and thyroid [14–18]. The largest series of Pax-8 stained whole-tissue sections of primary lung carcinoma showed that strong and diffused staining for Pax-8 was not observed in primary lung carcinoma, except in 5 of the 418 (1.2%) cases of large cell neuroendocrine carcinomas with a weak to moderate staining [19]. In the metastatic cases, both Tg and Pax-8 were negative for metastatic lung adenocarcinomas. Furthermore, our results also showed that Pax-8 had relatively higher sensitivity than that of Tg in metastatic thyroid carcinomas (95.1% vs. 63.4%). As there were issues with immunohistochemical staining for Tg in case of thyroid metastasis from lung carcinoma [20], Pax-8 may be more useful for the differential diagnosis of lung carcinoma and thyroid carcinoma.

Morphological features of thyroid carcinoma and lung adenocarcinoma are often distinctive in most cases. However, the morphology-based diagnosis of carcinomas was considered challenging sometimes, and IHC has been preferred after its introduction [21]. When a metastatic thyroid carcinoma showed patterns of papillary, micropapillary, solid, a lack of colloids, and was poorly differentiated we could not easily differentiate it from lung adenocarcinoma. The combined expression of napsin A, TTF-1, and CK7 in these tumors might also misguide a pathologist to diagnose thyroid carcinomas as lung adenocarcinoma especially in the case of a biopsy from a lung puncture without history. Chernock et al. described a misdiagnosis of metastatic thyroid case with an expression of TTF-1 and napsin A in lung in their reviewed cases [11]. Ito et al. recently described a poorly differentiated lung adenocarcinoma metastasize to the thyroid gland [22]. There were also cases reported previously regarding tumor to tumor metastasis among the carcinomas of lung and thyroid origin [23–25], increasing the diagnostic confusion. As the prognosis and treatment of thyroid carcinoma and lung adenocarcinoma are absolutely different; thus, to avoid a misdiagnosis which may lead to incorrect therapy, we recommended that a pathologic diagnosis among carcinomas from thyroid or lung origins should be based on clinical history, morphological features and a panel of markers at least including Pax-8.

The correlation of clinicopathological parameters with napsin A expression in thyroid carcinomas has not been investigated till date. In the cases of PTC, napsin A was significantly correlated with lymph node metastasis after pathologic investigation ($p = 0.030$), which was an interesting finding. The probable reason is that napsin A was mainly expressed in the classic, diffuse sclerosis, tall cell and solid variants of PTC. Most of these variants are more aggressive than indolent variants such as the follicular variant. It has been reported that napsin A may be important in the carcinogenesis of lung cancer is correlated with prognosis [26]. In our observation, napsin A was not detected in any normal thyroid tissue or in the hyperplasia of the thyroid. Further studies are needed to elucidate the mechanisms of aberrant napsin A expression in thyroid carcinoma.

In summary, we reported the expression of napsin A in various subtypes of thyroid tumors of which most cases showed a focal and weak to moderate expression. The positive expression of napsin A and conjunctive markers of TTF-1 and CK7 in a thyroid carcinoma should not confused it with lung carcinomas. The use of Pax-8 and Tg in the marker panel is recommended in cases that are not easily distinguishable. The relationship between napsin A expression and lymph node metastasis in PTC was a novel finding and further investigation is needed to highlight the roles of napsin A in thyroid tumors.

Author Contributions J W contributed to pathological and statistical analyses, manuscript writing, performed immunohistochemistry (IHC) analysis, and participated in experimental design.

Y Z contributed to pathological and statistical analyses, performed IHC analysis.

R C and W G made contributions to acquisition of clinical data.

T D, Y G, and Y L prepared the slides and performed IHC staining.

Y P and Q Z reviewed hematoxylin and eosin-stained (H&E) slides and performed pathological analysis.

W S checked the IHC and H&E slides.

D L and B S conceived the study, participated in its design and coordination, performed IHC analysis, and helped to draft and edit the manuscript.

Funding Information This work was supported by the National Natural Science Foundation of China (Grant No. 81871860) and the Science and Technology Fund of Tianjin Bureau of Public Health (NO. 2014KZ084).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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