

# **Napsin A is frequently expressed in clear cell carcinoma of the ovary and endometrium**

(卵巣・子宮内膜明細胞腺癌は高頻度に Napsin A を発現する)

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## **Napsin A is frequently expressed in clear cell carcinoma of the ovary and endometrium**

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## **Abstract**

Napsin A is a reliable marker for pulmonary adenocarcinoma. Recent studies have reported napsin A expression in a subset of ovarian clear cell carcinomas (O-CCCs), endometrial (EM)-CCCs, and endometrioid carcinomas (EC). This study investigated the extent of napsin A expression in O- and EM-CCC, and compared the former with other non-mucinous ovarian carcinomas. A total of 89 ovarian and uterine carcinoma cases (22 O-CCC, 15 EM-CCC, 13 ovarian [O-]JECs, and 39 high-grade serous carcinoma [HGSC]) were evaluated for napsin A, thyroid transcription factor (TTF)-1, paired box (PAX)8, and cancer antigen (CA)125 expression by immunohistochemistry. Napsin A immunoreactivity was observed in 21/22 (95.5%) O-CCC and 10/15 (66.7%) EM-CCC cases, but was rare in O-EC (7.7%) and undetectable in HGSC. There was no TTF-1 expression in O-CCC, but expression was detected in 1/15 (6.7%) EM-CCC, 3/13 (23.1%) O-EC, and 2/39 (5.1%) HGSC cases. All 89 cases examined were positive for PAX8, while all ovarian and 80.0% of EM-CCC cases were positive for CA125. There were no Napsin A/TTF-1 double-positive cases except for one EM-CCC, in which cells had a focal expression pattern. All napsin A- and/or TTF-1-positive cases expressed PAX-8 and CA125. In conclusion, napsin A is frequently expressed in O- and EM-CCC, rarely in O-EC, and never in HGSC. These findings confirm the importance of using a panel of antibodies that includes napsin A, TTF-1, PAX8, and/or CA125 when evaluating metastatic carcinomas of unknown origin, especially when gynecologic and pulmonary adenocarcinomas are included in the differential diagnosis.

**Keywords:** Napsin A, clear cell carcinoma, immunohistochemistry

## 1. Introduction

Napsin A is an aspartic proteinase expressed in type II pneumocytes, alveolar macrophages, and renal tubular epithelial cells. It is considered as a reliable marker for primary pulmonary adenocarcinoma, being expressed in 76.0%–90.7% of cases, with a sensitivity of 65%–81% and specificity of 88%–100% [1–6]. Napsin A expression has also been reported in extrapulmonary carcinomas, including papillary renal cell (72.0%–87.5%) [7, 8], clear cell renal cell (10%–34%) [5, 9], and thyroid (5%–50%) carcinomas [6, 9].

Recent studies have reported napsin A expression in a subset of ovarian clear cell carcinomas (O-CCCs) (68.8%–100%) as well as in up to 10% of ovarian and 6.8%–10.0% of endometrial (EM) endometrioid carcinomas; in contrast, it is rarely found in high-grade serous carcinoma (HGSC) [8, 10–13]. Only two studies have examined napsin A expression in EM-CCC, with expression observed in 4/6 (66.7%) cases in one study and 43/49 (87.8%) cases in the other [11, 14]. The present study investigated the extent of napsin A expression in O-CCC and EM-CCC, and compared the former with other ovarian carcinomas, including endometrioid carcinoma (O-EC) and HGSC.

## 2. Materials and methods

The surgical pathology archives of the Chiba University Hospital between January 2009 and September 2013 were searched for ovarian carcinoma and EM-CCC. Hematoxylin and eosin-stained specimens were reviewed to confirm the histological tumor type based on the World Health Organization 2014 criteria. A total of 89 consecutive cases of surgically resected ovarian and endometrial carcinomas were selected for this study, including 22 O-CCC, 13 O-EC, 39 HGSC, and 15 EM-CCC (pure or predominant) cases. Ovarian mucinous carcinomas, ovarian mixed carcinomas, and metastatic or recurrent carcinomas were excluded.

Formalin-fixed, paraffin-embedded tumor tissue specimens were sectioned at a thickness of 4  $\mu\text{m}$ ; the sections were deparaffinized and immunohistochemistry was performed using antibodies against the following proteins: napsin A (clone TMU-Ad02; American Research Products, Waltham, MA, USA) at 1:100; thyroid transcription factor (TTF)-1 (clone 8G7G3/1; Dako, Nikko, Japan) at 1:200; paired box (PAX)8 (polyclonal; Proteintech, Chicago, IL, USA) at 1:200; and cancer antigen (CA)125 (Ov185:1; Nichirei Biosciences Inc., Tokyo, Japan) at 1:1. PT-Link (Dako) was used for pretreatment and the Auto-stainer Link48 (Dako) was used for immunostaining. Antigen retrieval was performed by incubating sections at low pH Target Retrieval Solution (Dako) for 20 min at 98°C.

Granular cytoplasmic staining for napsin A, nuclear staining only of any intensity for TTF-1 and PAX8, and membranous staining for CA125 were considered positive. The immunohistochemical expression was scored as a proportion of positive tumor cells as follows: 0 = negative, < 10% = rare, 10%–60% = focal, and > 60% = diffuse. Rare, focal, and diffuse staining were considered positive.

### 3. Results

The results of the immunohistochemical analysis are summarized in Table 1. Of 22 O-CCC cases, 21 (95.5%) were positive for napsin A; the staining pattern was diffuse in eight (36.4%), focal in 11 (50.0%), and rare in two (9.1%) cases. The staining intensity was high in all diffuse and focal specimens. One negative O-CCC case was of the oxyphilic type. There were no differences in napsin A expression between the papillary, tubular, tubulocystic, or solid architecture, or clear or hobnail cell types. Two of the 21 napsin A-positive O-CCC specimens had a napsin A-expressing adenofibroma component adjacent to the carcinoma. Seven of the 21 napsin A-positive O-CCCs had concurrent endometriosis that was napsin A-negative. Napsin A was expressed in one (7.7%) of 13 O-EC cases with rare positive cells, and in none of the 39 HGSC specimens. Of 15 EM-CCCs, 10 (66.7%) were positive for napsin A; the staining pattern was diffuse in one (6.7%), focal in five (33.3%), and rare in four (26.7%) of the cases. Seven of 15 EM-CCCs contained a minor endometrioid carcinoma component; three of the seven cases were napsin A-positive in both clear cell and endometrioid carcinoma components.

TTF-1 was negative in all of the 22 O-CCCs, but was expressed in three (23.1%) of 13 O-EC, two (5.1%) HGSC, and one (6.7%) of 15 EM-CCC cases, usually with rare or focal positive cells. PAX8 immunoreactivity was detected in all 89 cases examined, while all ovarian carcinomas and 12 of 15 EM-CCCs (80.0%) were positive for CA125, usually with diffuse positive cells. There were no napsin A/TTF-1 double-positive cases except for one EM-CCC, in which the expression of both markers was focal in positive cells. PAX-8 and CA 125 were expressed in all napsin A- and/or TTF-1-positive cases.

#### 4. Discussion

The results of this study showed that napsin A is frequently expressed in O-CCC (95.5%), less commonly in EM-CCC (66.7%), rarely in O-EC, and never in HGSC. Our findings in ovarian non-mucinous carcinomas are consistent with previous studies, in which napsin A overexpression has been observed almost exclusively in O-CCC [10–13], but only in up to 10% of O-EC and < 1% of HGSC cases [10–12]. The distinction between O-CCC and HGSC is important, since they differ in terms of chemotherapeutic response, risk of thromboembolic complications, pattern of recurrence, prognosis, and association with specific genetic syndromes (that is, O-CCC in young patients with Lynch syndrome and HGSC with germline *BRCA1* and/or *BRCA2* mutations). Although in most cases the diagnosis of O-CCC is straightforward, the distinction between O-CCC and HGSC can be challenging when foci mimicking the characteristic features of O-CCC with papillary architecture, clear cytoplasm, or hobnail cells are present in HGSC. Wilms' tumor protein 1, estrogen receptor, and hepatocyte nuclear factor 1b was found to be the most sensitive and specific combination of markers that can be used to differentiate O-CCC from HGSC [15]. The present data demonstrate that napsin A is an additional marker that is useful for O-CCC diagnosis. It is unclear why a higher frequency of napsin A expression (87.8%) was observed in a previous investigation of napsin A expression in EM-CCC [14] as compared to the current findings; additional studies with more cases are required to determine whether napsin A is expressed at similar frequencies in EM-CCC and O-CCC and to elucidate the role of napsin A in the development and progression of these carcinomas.

O-CCC may occur in association with endometriosis or, less often, with clear cell adenofibroma. It was found here that an adenofibroma but not an endometriosis component adjacent to O-CCC was also positive for napsin A. Napsin A was found to

be expressed in 13 cases of clear cell adenofibroma not associated with carcinoma; five of eight cases of endometriosis were associated with carcinoma, but all of the six endometriosis cases were associated with carcinoma [12]. Further studies are necessary to clarify the link between napsin A expression and carcinogenesis and the biological behavior of tumors, which could influence patient prognosis.

We also found that TTF-1—another reliable pulmonary adenocarcinoma marker routinely used in pathology practice—is rarely positive in non-mucinous ovarian carcinomas or in EM-CCCs. TTF-1 is a nuclear protein expressed in epithelial cells of the thyroid, type II pneumocytes, and Clara cells in the lung. Its expression has been reported in the majority of primary pulmonary adenocarcinomas (70.0%–82.5%) [1, 6, 16–18] and thyroid papillary carcinomas (97%–100%) [9, 19], and less often in other primary sites including the gastrointestinal tract, kidney, urinary bladder, and prostate and in gynecologic tumors [10, 11, 17, 18, 20–23]. One study reported that only a small fraction of pulmonary adenocarcinomas were TTF-1(-)/napsin A(-) (9.2%) or TTF-1(-)/napsin A(+) (8.3%), while the majority (79.2%) were double-positive for napsin A and TTF-1 [16]. In our study, only one EM-CCC specimen was napsin A/TTF-1 double-positive, and the expression of both markers was focal. In ovarian carcinoma, TTF-1 expression has been detected in 5.5%–37% of serous, 6.3%–33% of clear cell, and 3.6%–20% of endometrioid carcinomas [10, 11, 22, 23]. In one study evaluating both napsin A and TTF-1 expression in ovarian non-mucinous carcinomas, the frequency of napsin A/TTF-1 double-positive cases was 13.8% in O-CCC and 6.9% in O-EC, with rare or focal expression of both markers [10]; another study found no double-positive cases [11]. These findings indicate that immunoreactivity for both napsin A and TTF-1 can distinguish primary lung adenocarcinoma from metastatic ovarian or endometrial carcinoma in the lung.

Napsin A immunoreactivity in a CCC of ovarian or endometrial origin can

potentially be misinterpreted by pathologists as a pulmonary adenocarcinoma when encountered in small biopsy specimens from the lung or lymph nodes. Primary pulmonary adenocarcinomas have variable histological features, and distinguishing them from metastatic adenocarcinomas based on morphology alone can be very challenging, especially with small biopsy specimens. Furthermore, O- or EM-CCCs that have papillary or tubular architecture can resemble papillary- or acinar-type pulmonary adenocarcinomas. Nonetheless, differentiating between primary and metastatic adenocarcinomas is important from a therapeutic standpoint. The lungs are a common site of metastasis from extrapulmonary carcinomas, and recently developed tyrosine kinase inhibitors targeting specific molecular alterations have achieved successful clinical outcomes almost exclusively in patients with adenocarcinoma of pulmonary origin but not those resulting from metastasis from other organs [24].

The current study confirmed that inclusion of gynecologic tract-specific markers such as PAX8 and/or CA125 in addition to TTF-1 in an immunohistochemical panel should help to resolve this ambiguity. Indeed, we observed one case of EM-CCC (not included in the present study) that had metastasized to the lung, in which diagnosis of a small lung biopsy based on morphology alone was challenging. Immunohistochemical detection of napsin A, PAX8, and CA125, but TTF-1 in both endometrial and lung tumor specimens, along with the patient's history of EM-CCC five years prior and similarities in morphology confirmed the diagnosis. PAX 8 is one of nine members of the PAX family of transcription factors that plays an important role in the development of a variety of organs, including those derived from the Wolffian and Müllerian ducts as well as the thyroid, kidney, central nervous system, inner ear, and eye [25]. PAX8 expression is prevalent in most non-mucinous ovarian and endometrial carcinomas (up to 100%) [26], as well as renal cell and thyroid carcinomas, but is always negative in carcinomas of the lung and adenocarcinomas of the breast, prostate, stomach, colon,

bladder, salivary gland, and bile duct. CA125 is expressed in up to 61% of tumors in the female genital tract, and less frequently in adenocarcinomas of the pancreas (up to 82%), bile ducts (up to 56%), and lung (up to 20%) [27, 28]. In the female genital tract, while constant CA125 expression has been reported in ovarian and endometrial serous and endometrioid carcinomas as well as in EM-CCC, some studies have shown slightly less positive immunoreactivity in ovarian CCC [29, 30]. In our study, although all ovarian and the majority of EM-CCC cases were positive for CA125, those with a diffuse staining pattern were relatively rare.

In conclusion, napsin A was frequently expressed in O-CCC, and was less common in EM-CCC, rare in O-EC, and was never observed in HGSC. TTF-1 was not expressed in O-CCC, but was occasionally detected in EM-CCC, O-EC, and HGSC. PAX8 and CA125 were expressed in almost all cases of O-CCC, EM-CCC, O-EC, and HGSC. These findings confirm the importance of using a panel of antibodies that includes napsin A, TTF-1, PAX8, and/or CA125 to evaluate metastatic carcinomas of unknown origin in which gynecologic and pulmonary adenocarcinomas are included in the differential diagnosis.

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**Table 1.** Immunohistochemical analysis of napsin A, TTF-1, PAX8, and CA125 expression

O-CCC, ovarian clear cell carcinoma; EM-CCC, endometrial clear cell carcinoma;

O-EC, ovarian endometrioid carcinoma; HGSC, high-grade serous carcinoma.

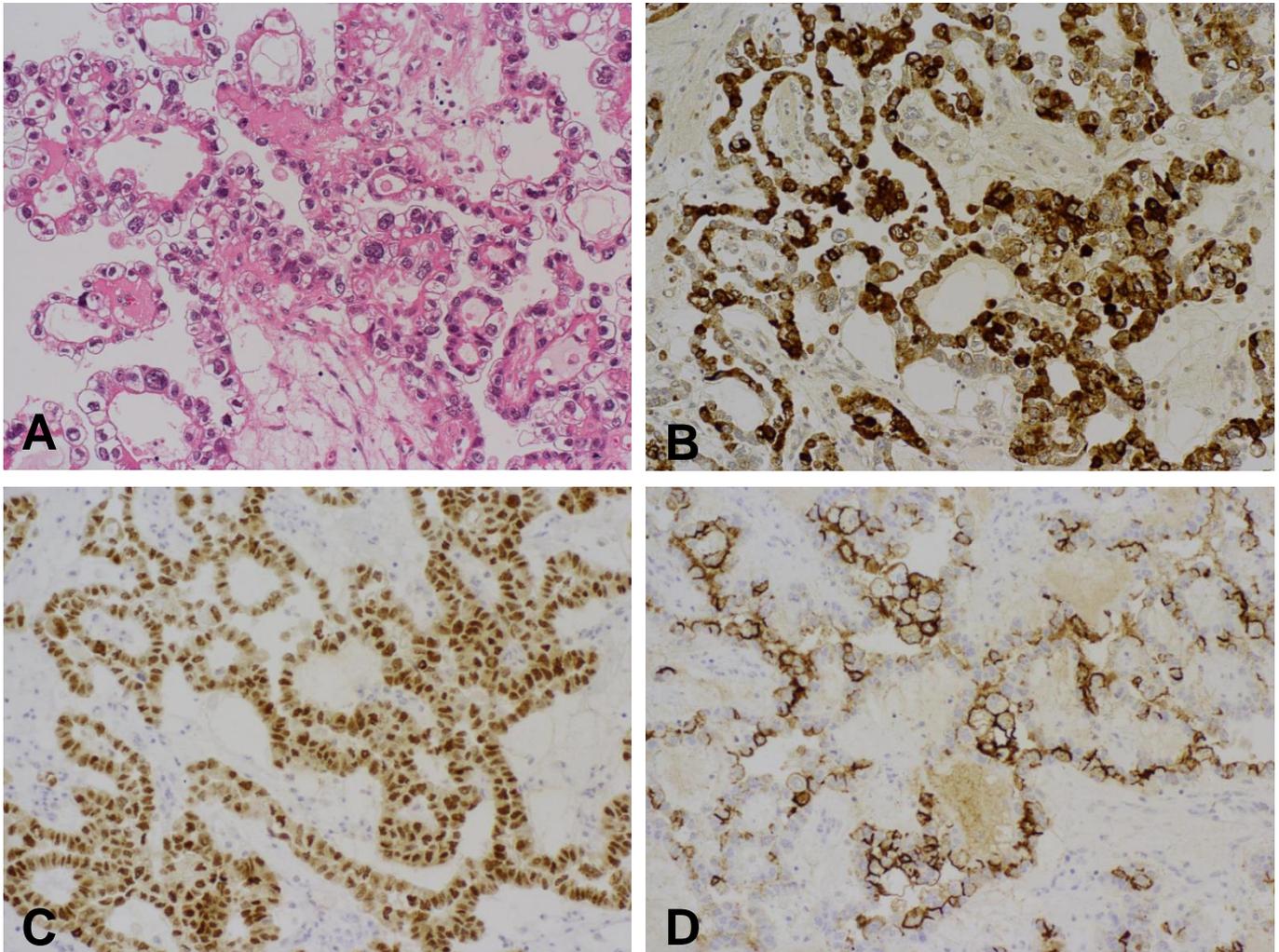
**Figure 1.** Representative case of O-CCC. A: Hematoxylin and eosin staining. B: Diffuse napsin A expression; granular cytoplasmic staining for napsin A was considered positive. C: Diffuse nuclear staining for PAX8. D: Membranous staining for CA125.

**Figure 2.** Representative case of EM- CCC. A: Hematoxylin and eosin staining. B: Diffuse napsin A expression.

**Table 1.**

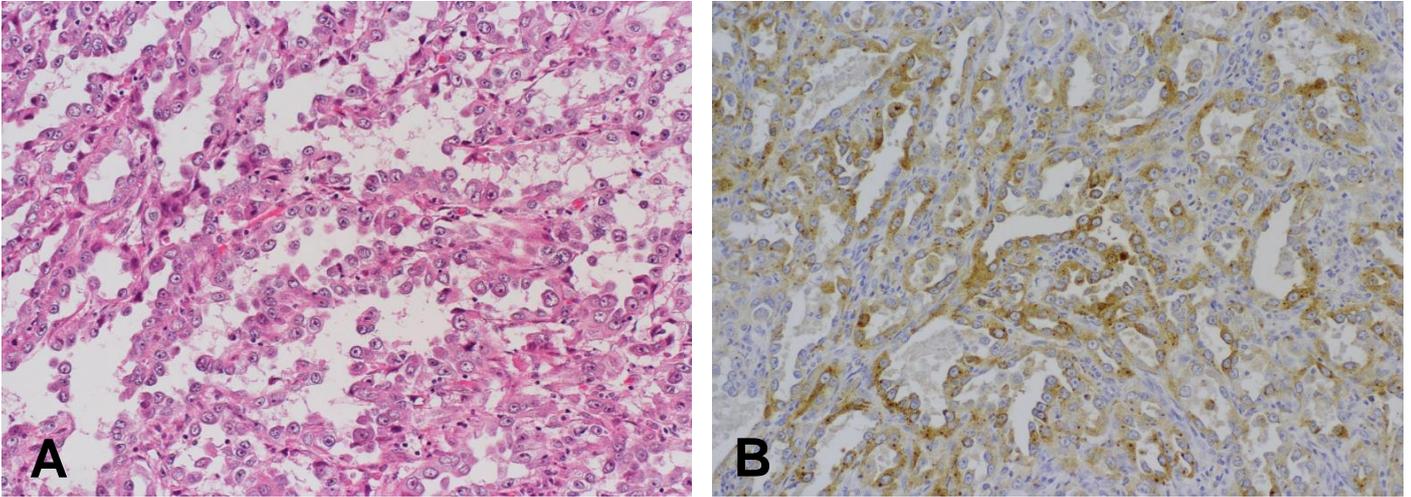
	O-CCC (n=22)	EM-CCC (n=15)	O-EC (n=13)	HGSC (n=39)
<b>Napsin A</b>				
negative	1 (4.5%)	5 (33.3%)	12 (92.3%)	39 (100%)
rare	2 (9.1%)	4 (26.7%)	1 (7.7%)	0
focal	11 (50.0%)	5 (33.3%)	0	0
diffuse	8 (36.4%)	1 (6.7%)	0	0
<b>TTF-1</b>				
negative	22 (100%)	14 (93.3%)	10 (76.9%)	37 (94.9%)
rare	0	0	1 (7.7%)	2 (5.1%)
focal	0	1 (6.7%)	2 (15.4%)	0
diffuse	0	0	0	0
<b>PAX8</b>				
negative	0	0	0	0
rare	0	1 (6.7%)	1 (7.7%)	0
focal	0	0	1 (7.7%)	5 (12.8%)
diffuse	22 (100%)	14 (93.3%)	11 (84.6%)	34 (87.2%)
<b>CA125</b>				
negative	0	3 (20.0%)	0	0
rare	2 (9.1%)	1 (6.7%)	1 (7.7%)	1 (2.6%)
focal	6 (27.3%)	5 (33.3%)	1 (7.7%)	2 (5.1%)
diffuse	14 (63.6%)	6 (40.0%)	11 (84.6%)	36 (92.3%)

**Figure 1.**



Representative case of O-CCC. A: Hematoxylin and eosin staining. B: Diffuse napsin A expression; granular cytoplasmic staining for napsin A was considered positive. C: Diffuse nuclear staining for PAX8. D: Membranous staining for CA125.

**Figure 2.**



Representative case of EM- CCC. A: Hematoxylin and eosin staining.  
B: Diffuse napsin A expression.

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