

Original Article

Role of napsin A immunohistochemical staining in differentiating ovarian clear cell carcinoma from other ovarian epithelial tumors

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Abstract: Clear cell carcinoma, which accounts for 10% of all ovarian cancers, has the poorest prognosis among ovarian cancer subtypes because of chemoresistance. Making a differential diagnosis between clear cell carcinoma and other ovarian epithelial tumors is important for determining the appropriate treatment modality. Napsin A, a member of the peptidase A1 family, is an aspartic proteinase that is expressed in normal lung and kidney tissues. We investigated the expression of napsin A in ovarian clear cell carcinoma using immunohistochemistry, and determined its usefulness for differentiating among primary ovarian epithelial tumors. A total of 36 ovarian cancer cases (16 primary clear cell carcinoma, 13 primary serous carcinoma, and 7 primary ovarian endometrioid carcinoma) with definitive diagnoses made between 1998 and 2015 were including in the study. All cases were evaluated for the immunoexpression of napsin A. In primary ovarian clear cell carcinoma cases, 3 (18.7%) stained negative for napsin A, whereas extensive strong cytoplasmic expression was observed in 13 (81.3%). No napsin A expression was detected in primary ovarian endometrioid and serous carcinoma samples. In conclusion, napsin A expression is a highly sensitive and specific marker that can be used to differentiate ovarian clear cell carcinoma from other tumors pathologically. It is expressed at high levels in ovarian clear cell carcinoma but not in other epithelial tumors.

Keywords: Ovary, clear cell carcinoma, napsin A

Introduction

Ovarian cancers are associated with high a mortality and are among the 10 most common cancers in females worldwide [1]. It is also the leading cause of gynecologic cancer-related deaths. The incidence of ovarian cancer increases with age, and the mean age at onset is 63 years [1]. The incidence of malignant ovarian neoplasia is 30% in the postmenopausal period, compared to 7% in the premenopausal period [2]. The factors that increase the risk of ovarian cancer include age, parity, and family history [3]. However, a substantial number of ovarian cancer cases are sporadic and occur without any risk factor.

Because the ovaries contain different embryologic tissues, there are many different types of complex ovarian tumors. About 59% of overall ovarian neoplasia and 85-90% of ovarian cancers arise from epithelial tissue [4]. Ovarian

epithelial tumor subtypes include serous, mucinous, endometrioid, clear cell, Brenner, and seromucinous tumors [5]. Mucinous tumors are the most prevalent subtype. Serous carcinomas, which have malignant character, can be divided into two groups: low grade and high grade [5]. Endometrioid carcinomas, the second most prevalent subtype, histologically resemble endometrioid adenocarcinomas and are accompanied by primary endometrial cancer in 15-20% of cases [6]. Clear cell carcinoma, which accounts for 10% of the overall number of ovarian cancers, usually occurs between the fifth and seventh decades of life. Clear cell carcinoma is most frequently accompanied by endometriosis and is histologically characterized by hobnail cells with hyperchromatic nuclei located in the upper margin of the cytoplasm [5]. Clear cell carcinomas have the poorest prognosis among all ovarian cancer subtypes because they are chemoresistant. Therefore, differentiating clear cell carcinomas from other

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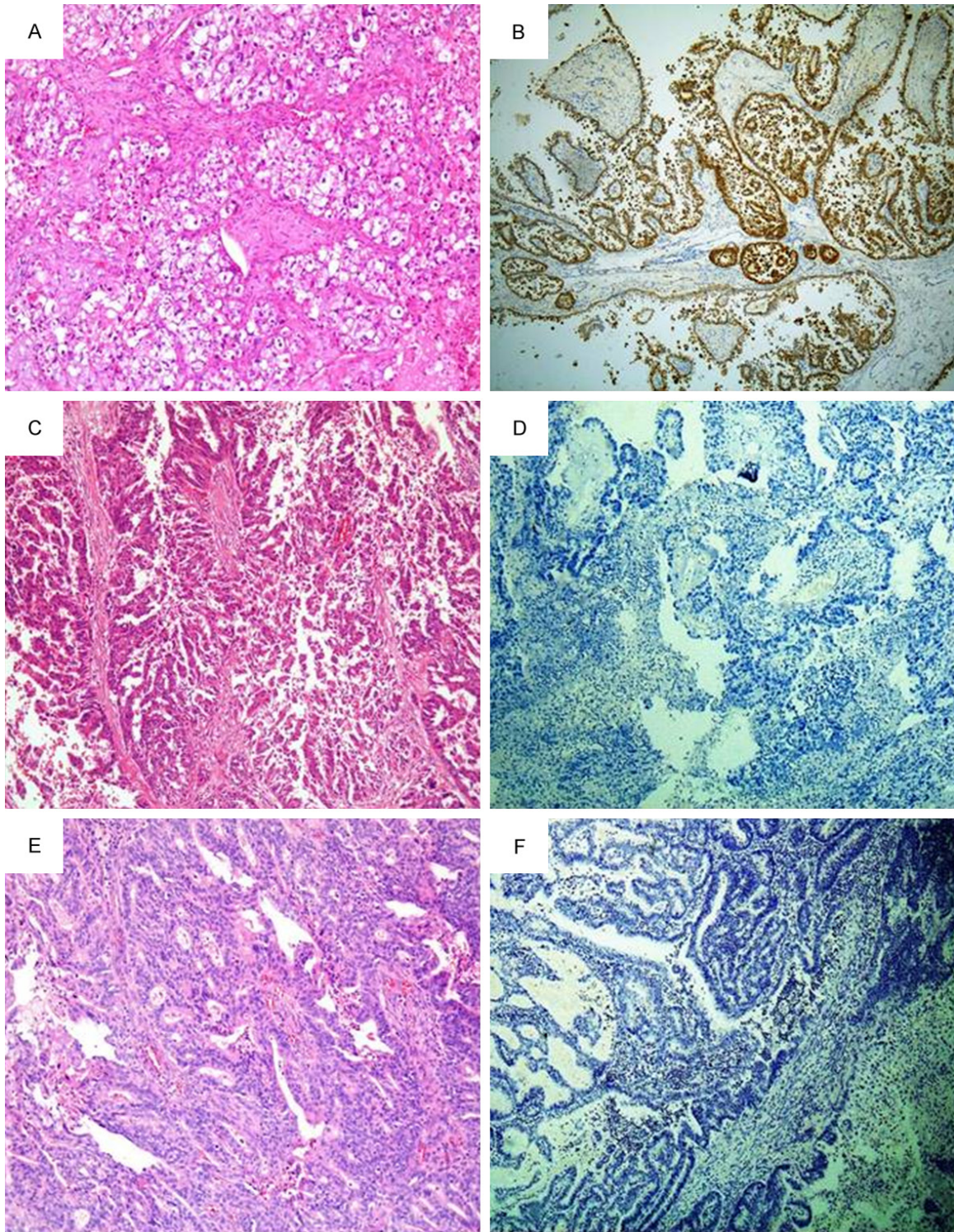


Figure 1. A. Ovarian clear cell carcinoma characterized by hobnail cells with hyperchromatic nuclei located in the upper margin of the cytoplasm (H&E; magnification $\times 100$). B. Strong cytoplasmic napsin A expression in ovarian clear cell carcinoma tumor cells (napsin A; $\times 40$). C. Ovarian serous carcinoma with papillary clusters of serous epithelial cells in the ovarian stroma (H&E; $\times 100$). D. Negative napsin A staining in serous carcinoma (napsin A; $\times 40$). E. Endometrioid carcinoma with a villoglandular pattern that histologically resembles endometrioid adenocarcinoma of the endometrium (H&E; $\times 100$). F. No napsin A immunoreactivity reactivity in endometrioid carcinoma (Napsin A; $\times 40$).

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ovarian epithelial tumors is of great importance for determining the appropriate treatment modality [7].

Napsin A is an aspartic proteinase that is a member of the peptidase A1 family; it is expressed in normal lung and kidney tissues. Previous studies have demonstrated that lung adenocarcinomas exhibit positive immunohistochemical staining for napsin A with high sensitivity (59-100%) and specificity (88-94%) [8]. Napsin A expression has also been reported in extrapulmonary carcinomas such as papillary renal cell, clear renal cell, and thyroid carcinomas [8-10]. Furthermore, napsin A expression can be used to differentiate ovarian clear cell carcinomas from other ovarian epithelial tumors [11, 12]; specifically, it is expressed in a higher percentage of ovarian clear cell carcinomas (99.5-100%) compared to other ovarian cancers [11, 12].

We evaluated the expression of napsin A in ovarian clear cell carcinoma and investigated its utility for differentiating among primary ovarian epithelial tumors.

Materials and methods

Subjects and tissue samples

Thirty-six ovarian tumor cases from patients who underwent surgical procedures between 1998 and 2005 and had been pathologically diagnosed as primary ovarian carcinoma were obtained. All cases were evaluated again by two pathologists and the diagnoses were reviewed according to the 2014 WHO classification [5]. All cases had a definitive diagnosis: 16 primary clear cell carcinoma, 13 primary serous carcinoma, and 7 primary endometrioid carcinoma. The Medical Studies Ethics Committee approved the current study (date 21.07.2015 and no. 2015-14/5).

Immunohistochemical staining

The most diagnostic single block was selected from formaldehyde-fixed, paraffin-embedded preparations from the ovaries of all 36 cases. For immunohistochemical analysis, 4 µm sections were obtained from these blocks and stained with napsin A clone IP64 (1:400, Novocastra, Newcastle, United Kingdom) antibodies. After incubation for 40 min the slides were processed with a Leica Bond-Max auto-

staining device. Lung tissue was used as the external control block for the napsin A antibody.

Staining evaluation

In staining evaluations, cytoplasmic napsin A staining was considered positive. The extent and intensity of staining were assessed subjectively. Intensity was scored as 1 (weak), 2 (intermediate), and 3 (strong). Extent was evaluated as 1 (positive staining in <25% of the cells), 2 (positive in 25-50% of cells), and 3 (positive in >50% of cells).

Statistical analysis

Data that exhibited a normal distribution were analyzed using Shapiro-Wilk tests. Between groups comparisons were performed by using Kruskal Wallis or Fisher-Freeman Halton test. Chi-square tests were used to compare categorical variables between two groups. The correlation between variables was analyzed using Spearman's correlation coefficient. Statistical significance was set at $P < 0.05$ and SPSS 20.0 was used for performing statistical analysis.

Results

Clinical findings

The histopathological diagnosis of the 36 tumors indicated 16 primary ovarian clear cell carcinomas, 13 primary serous carcinomas, and 7 primary ovarian endometrioid carcinomas. The mean age of the patients with ovarian clear cell carcinoma, ovarian endometrioid carcinoma, and serous carcinoma was 60 (range, 48-83), 43.8 (30-56), and 58.5 (47-69), respectively.

Immunohistochemical findings

In primary ovarian clear cell carcinoma cases, 3 (18.7%) stained negative for napsin A, whereas extensive strong cytoplasmic expression was observed in 13 (81.3%). No napsin A expression was detected in primary ovarian endometrioid and serous carcinoma samples.

The staining extent score was 2+ in 3 (18.7%) and 3+ in 10 (62.5%) cases with primary ovarian clear cell carcinoma (**Figure 1**). The distribution of napsin A-positive cases according to the staining intensity was as follows: 1 in one case

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(6.3%), 2 in five cases (31.2%), and 3 in seven cases (43.7%).

Napsin A staining significantly differentiated primary ovarian clear cell carcinoma from serous and endometrioid carcinomas ($P < 0.001$). The sensitivity and specificity of napsin A staining for differentiating among the tumor types was 81.25% and 100%, respectively, with a positive predictive value of 100% and a negative predictive value of 86.96%.

Discussion

Gynecological malignancies are one of the most important causes of cancer-related morbidity and mortality in females following breast cancer. Therefore, identifying the incidence, determining the risk factors, investigating the etiopathogenesis, and developing appropriate strategies for the prevention and treatment of gynecologic cancers is of great importance [13]. The different ovarian epithelial tumor subtypes include serous, mucinous, endometrioid, clear cell, Brenner, and seromucinous tumors [5]. According to the World Health Organization (WHO) definition, ovarian clear cell carcinoma is a malignant epithelial tumor that is composed of clear, eosinophilic, and hobnail cells that show tubulocystic, papillary, and solid patterns [5].

Ovarian primary clear cell carcinoma occurs between the fifth and seventh decades of life and is bilateral in 3% of cases [14]. Chan [15] investigated a large series of 1411 ovarian clear cell cancer cases, and compared ovarian clear cell carcinoma to other ovarian epithelial tumors. The mean age of patients with other ovarian epithelial tumors was 65 years, compared to <55 years for primary ovarian clear cell carcinoma. In our study, the mean age was 60 years, which differs somewhat from the literature; this difference could be explained by the limited patient number in our study.

Patients with ovarian primary clear cell carcinoma have the poorest prognosis among ovarian cancer subtypes because they are chemoresistant. Therefore, differentiating between clear cell carcinoma and other ovarian epithelial tumors is of great importance to identify the appropriate treatment modality [7]. The differential diagnosis of clear cell carcinomas includes endometrioid, serous, and other superficial epithelial malignant tumors [5]. The cyto-

plasm may be uniformly clear due to a high glycogen content in the squamous component of endometrioid carcinomas that show secretory changes, the glands floored by columnar cells containing subnuclear and supranuclear vacuoles (which resemble the early secretory endometrium), and some typical endometrioid carcinomas. Clear cells may also be detected in mixed epithelial borderline tumors; clear cell carcinoma should be considered when these neoplasms contain hobnail cells accompanied by endometriosis [16].

Immunohistochemical staining is used to make differential diagnoses in cases when morphology is insufficient to differentiate ovarian primary clear cell carcinoma from other ovarian endometrioid and serous carcinomas. In one study, ovarian clear cell carcinomas stain positive for Pax8 (99%), p53 (12%), the estrogen receptor (ER; 13%), and the progesterone receptor (PR; 6%); however, this immunohistochemical panel was incapable of differentiating ovarian clear cell carcinomas from other malignant ovarian epithelial carcinomas [5].

Some previous studies have focused on differentiating ovarian primary clear cell carcinomas from other epithelial ovarian tumors. These studies have demonstrated that immune determinants such as hepatocyte nuclear factor 1 beta (HNF-1 β), napsin A, and alpha-methylacyl-coenzyme A racemase (AMACR) might be helpful. Qing [17] determined that HNF1 β had a high sensitivity (100%) and low specificity (54.4%) for ovarian clear cell carcinomas whereas napsin A had a high sensitivity and specificity (97.4% and 91.2%, respectively); therefore, napsin A is a marker more specific marker than HNF1 β . Fadare [18] found that AMACR had a low sensitivity (63%) and high specificity (99%) for identifying ovarian clear cell carcinomas; in contrast, napsin A had a moderate sensitivity (82%) and high specificity (99%). We found that the sensitivity, specificity, positive predictive value, and negative predictive value of napsin A staining were 81.25%, 100%, 100%, and 86.96%, respectively, for differentiating ovarian clear cell carcinomas from endometrioid and serous carcinomas, consistent with the literature.

Yamashita [11] investigated napsin A-positive staining in 195 ovarian tumor cases between 1986 and 2013: 86 cases of ovarian clear cell

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tumor, 13 ovarian clear cell adenofibromas, 30 serous adenocarcinomas, 11 serous adenomas or borderline tumors, 19 ovarian endometrioid adenocarcinomas, 22 mucinous or borderline tumors, and 10 mucinous adenocarcinomas. Positive napsin A staining was observed in 71 (83%) clear cell carcinoma cases and all 13 (100%) clear cell adenofibroma cases but none of the remaining non-clear cell carcinoma epithelial tumors. They recommended including napsin A in panels used to differentiate among ovarian epithelial tumors. Our results are consistent with these results. Specifically, all cases of serous and endometrioid carcinomas stained negative for napsin A, whereas 81.3% of clear cell carcinomas stained positive.

Skirnisdottir [19] studied the potential of napsin A for differentiating between ovarian clear cell carcinoma and other epithelial carcinomas. They identified positive napsin A staining in 12 of 16 (80%) clear cell carcinoma cases; the percent positive staining was significantly different compared to other serous and endometrioid tumors ($P < 0.001$).

Another study used immunohistochemistry to investigate the expression of napsin A, TTF-1, PAX-8, and CA125 in 22 cases of ovarian clear cell carcinoma, 15 cases of endometrial clear cell carcinoma, 13 cases of ovarian endometrioid carcinoma, 39 cases of high-degree serous carcinoma, and 22 cases of endometrioid endometrial carcinoma [20]. Napsin A-positive immunoreactivity was observed in 21 (95.5%) ovarian clear cell carcinoma and 10 (66.7%) endometrial clear cell carcinoma cases. However, positive staining was observed in only 7.7% of ovarian endometrioid carcinoma and 4.5% of endometrial endometrioid carcinoma cases. No cases of high-degree serous carcinoma exhibited positive napsin A staining. Therefore, napsin A is frequently expressed in ovarian clear cell carcinoma and endometrial clear cell carcinoma, rarely expressed in ovarian endometrioid carcinoma and endometrioid endometrial carcinoma, and never expressed in high-degree serous carcinoma. Consistent with this, we found napsin A-positive staining in primary ovarian endometrioid carcinoma and serous carcinoma cases.

Conclusion

In conclusion, the immunohistochemical antibodies that can be used to differentiate ovarian

primary clear cell carcinoma from other primary ovarian epithelial tumors are still being investigated. The sensitivity and specificity of the current antibodies are not high enough, and no antibodies that can be used to diagnose ovarian clear cell carcinoma are available. Therefore, new antibodies that are highly sensitive and specific are needed. The current study suggests that napsin A is a highly sensitive and specific marker that can be used in pathological immunohistochemistry to differentiate ovarian clear cell carcinoma from other tumors.

Disclosure of conflict of interest

None.

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References

- [1] Siegel R, Naishadham D, Jemal A. Cancer statistics. *CA Cancer J Clin* 2013; 63: 11-30.
- [2] Fiorca JV, Roberts WS. Screening for ovarian cancer. *Cancer Control* 1996; 3: 120-9.
- [3] Whittemore AS, Balise RR, Pharoah PD, Dicioccio RA, Oakley-Girvan I, Ramus SJ, Daly M, Usinowicz MB, Garlinghouse-Jones K, Ponder BA, Buys S, Senie R, Andrulis I, John E, Hopper JL, Piver MS. Oral contraceptive use and ovarian cancer risk among carriers of BRCA1 or BRCA2 mutations. *Br J Cancer* 2004; 91: 1911-5.
- [4] Berek SJ. Berek and Novak's Gynecology. 14th edition. Philadelphia: Lippincott Williams & Wilkins; 2007: 1457-9.
- [5] Kurman RJ, Carcangiu ML, Herrington CS, Young RH. WHO Classification of Tumours of Female Reproductive Organs, 4th edition. Lyon: IARC; 2014. PP. 15-41.
- [6] Irving JA, Catasús L, Gallardo A, Bussaglia E, Romero M, Matias-Guiu X, Prat J. Synchronous endometrioid carcinomas of the uterine corpus and ovary: alterations in the beta-catenin (CTNNB1) pathway are associated with independent primary tumors and favorable prognosis. *Hum Pathol* 2005; 36: 605-19.
- [7] Sugiyama T, Kamura T, Kigawa J. Clinical characteristics of clear cell carcinoma of the ovary: a distinct histologic type with poor prognosis and resistance to platinum-based chemotherapy. *Cancer* 2000; 88: 2584-9.
- [8] Kadivar M, Boozari B. Applications and limitations of immunohistochemical expression of "Napsin-A" in distinguishing lung adenocarcinoma from adenocarcinomas of other organs.

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- Appl Immunohistochem Mol Morphol 2013; 21: 191-5.
- [9] Mukhopadhyay S, Katzenstein AL. Comparison of monoclonal napsin A, polyclonal napsin A, and TTF-1 for determining lung origin in metastatic adenocarcinomas. *Am J Clin Pathol* 2012; 138: 703-11.
- [10] Bishop JA, Sharma R, Illei PB. Napsin A and thyroid transcription factor-1 expression in carcinomas of the lung, breast, pancreas, colon, kidney, thyroid, and malignant mesothelioma. *Hum Pathol* 2010; 41: 20-5.
- [11] Yamashita Y, Nagasaka T, Naiki-Ito A, Sato S, Suzuki S, Toyokuni S, Ito M, Takahashi S. Napsin A is a specific marker for ovarian clear cell adenocarcinoma. *Modern Pathology* 2015; 28: 111-7.
- [12] Iwamoto M, Nakatani Y, Fugo K, Kishimoto T, Kiyokawa T. Napsin A is frequently expressed in clear cell carcinoma of the ovary and endometrium. *Hum Pathol* 2015; 46: 957-62.
- [13] Kurman RJ, Shih IeM. The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. *Am J Surg Pathol* 2010; 34: 433-43.
- [14] Fox H. *Obstetrical and Gynaecological Pathology, Volume 1* Third ed. Edinburgh, London, Melbourne and New York, Churchill Living Stone 1987; 600-8.
- [15] Chan JK, Teoh D, Hu JM, Shin JY, Osann K, Kapp DS. Do clear cell ovarian carcinomas have poorer prognosis compared to other epithelial cell types? A study of 1411 clear cell ovarian cancers. *Gynecol Oncol* 2008; 109: 370-6.
- [16] Shevchuk MM, Winkler-Monsanto B, Fenoglio C M, Richart RM. Clear cell carcinoma of the ovary: A clinicopathologic study with review of the literature. *Cancer* 1981; 47: 1344-51.
- [17] Li Q, Zeng X, Cheng X, Zhang J, Ji J, Wang J, Xiong K, Qi Q, Huang W. Diagnostic value of dual detection of hepatocyte nuclear factor 1 beta (HNF-1 β) and napsin A for diagnosing ovarian clear cell carcinoma. *Int J Clin Exp Pathol* 2015; 8: 8305-10.
- [18] Fadare O, Zhao C, Khabele D, Parkash V, Quick CM, Gwin K, Desouki MM. Comparative analysis of Napsin A, alpha-methylacyl-coenzyme A racemase (AMACR, P504S), and hepatocyte nuclear factor 1 beta as diagnostic markers of ovarian clear cell carcinoma: an immunohistochemical study of 279 ovarian tumours. *Pathology* 2015; 47: 105-11.
- [19] Skirnisdottir I, Bjersand K, Akerud H, Seidal T. Napsin A as a marker of clear cell ovarian carcinoma. *BMC Cancer* 2013; 13: 524.
- [20] Iwamoto M, Nakatani Y, Fugo K, Kishimoto T, Kiyokawa T. Napsin A is frequently expressed in clear cell carcinoma of the ovary and endometrium. *Hum Pathol* 2015; 46: 957-62.