Annals of Diagnostic Pathology 48 (2020) 151582

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

Original Contributions

Evaluation of MTAP immunohistochemistry loss of expression in ovarian serous borderline tumors as a potential marker for prognosis and progression

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ARTICLE INFO

Keywords:
Serous borderline tumor
Low-grade serous carcinoma
MTAP
CDKN2A
Chromosome 9p21

ABSTRACT

Introduction: Serous borderline tumors (SBT) are the most common subtype of ovarian borderline tumors with excellent clinical course. However, they can recur or progress to low-grade serous carcinoma (LGSC) in a small proportion of the cases. Beside BRAF and KRAS mutations, copy number alterations (CNA), particularly loss of chromosome 9p21 locus which results in deletion of genes CDKN2A and MTAP, have been suggested to be involved in disease progression. MTAP immunohistochemistry recently has been introduced for mesothelioma as a reliable surrogate marker for the homozygous deletion of chromosome 9p21 locus. Therefore, in the current study, we aimed to evaluate the MTAP loss of expression in serous borderline tumors and low-grade serous carcinomas to identify if it can be used as a marker for prognosis and progression.

Method: Eighty-four total cases of ovarian serous lesions, including 21 cases of serous cystadenomas, 21 cases of serous borderline tumors, 12 cases of low-grade serous carcinomas and 30 cases of high-grade serous carcinomas were selected. MTAP immunohistochemistry was performed on the representative blocks and cytoplasmic staining was used for interpretation. The cases were labeled as positive (retained) if MTAP showed cytoplasmic granular staining and negative (loss of expression) if negative cytoplasmic staining was observed in the presence of positive internal control.

Result: Ten of 21 cases of serous borderline tumors showed loss of MTAP expression (48%). Among those, 7 cases were bilateral, 2 cases had micropapillary features, one case had supraclavicular and cervical lymph node involvement by serous borderline tumor and 2 cases had progression to low-grade serous carcinoma, including one of micropapillary tumors. Also 8 out of 12 cases of LGSCs showed MTAP loss of expression (66%). Only 4 of 30 cases of high-grade serous carcinomas (13%) and none of the serous cystadenoma cases showed loss of expression of MTAP.

Conclusion: To our knowledge, this is the first description of MTAP immunohistochemistry in serous borderline tumors and low-grade serous carcinomas. Our study was limited due to small sample size. However, it showed an association between MTAP loss of expression and adverse clinical behavior in ovarian serous borderline tumors. This supports the role for further investigations in larger series to evaluate the role of MTAP stain as a prognostic marker in these neoplasms.

1. Introduction

Serous borderline tumors (SBT), also known as “atypical proliferative serous tumor”, are the most common subtype of ovarian borderline tumors. They usually affect women at younger age and have clinical behavior intermediate between benign and malignant tumors. Although most of the SBTs have excellent clinical course, they can recur or progress to low-grade serous carcinoma (LGSC) in 4–7% of the patients. There are some histologic features associated with increased likelihood of their recurrence or progression, including micropapillary architecture, bilateral ovarian involvement and presence of extra-ovarian disease, particularly in the form of invasive implants [1-3].

Low-grade serous carcinomas (LGSC) are indolent invasive tumors with tendency to occur at younger age and comprise 10% of ovarian serous carcinomas. They are in continuum with SBTs and have different molecular pathway and clinical behavior than high-grade serous

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https://doi.org/10.1016/j.anndiagpath.2020.151582

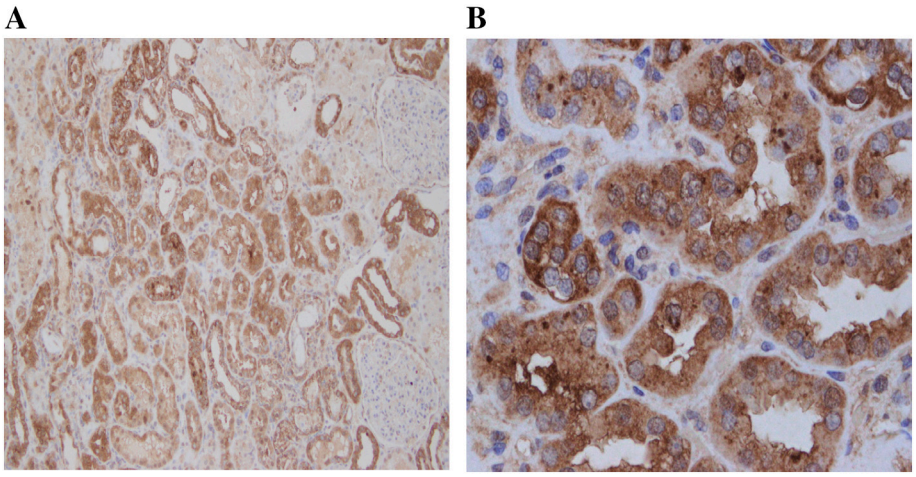

Fig. 1. Renal tubules of normal kidney tissue were used as MTAP immunohistochemistry positive control (A). MTAP was interpreted as positive in the presence of granular cytoplasmic staining (B).

carcinomas. Despite their low grade morphology, it is not uncommon for these tumors to demonstrate aggressive clinical course with more resistance to chemotherapy [4,5].

Although KRAS and BRAF mutations have been described in SBTs and LGSCs as the most common somatic mutations [6], the molecular profiling of these lesions has suggested that copy number alterations (CNA), specifically loss of chromosome 9p and homozygous deletion of CDKN2A locus also may play role in progression of SBTs to LGSCs [7]. CDKN2A gene, located on chromosome 9p21 locus encodes significant tumor suppressor proteins including p16 (INK4A) and the p14 (ARF) [8]. This homozygous deletion of CDKN2A locus usually results in co-deletion of the adjacent genes, including methylthioadenosine phosphorylase (MTAP) in a large number of the cases [9]. MTAP has an important role in metabolism of polyamine and salvage of adenine and methionine and there are some evidence now that it also acts as a tumor suppressor itself [10]. MTAP immunohistochemistry (IHC) recently has been introduced for mesothelioma as a marker for detection of homozygous deletion of chromosome 9p21. Several studies on mesothelioma cases have shown that MTAP IHC has high sensitivity and specificity for detection of this homozygous deletion and therefore can be used as a reliable surrogate marker for this genetic alteration [11-13].

In the current study, we aimed to evaluate the loss of expression of MTAP immunohistochemistry in ovarian serous borderline tumors and low-grade serous carcinomas as a surrogate marker for homozygous deletion of chromosome 9p21to identify if it can be used as a marker for prognosis and progression of SBTs.

2. Method

2.1. Case selection

For the conduct of this study, an approval was obtained from the Institutional Review Board at David Geffen (IRB# 20-000447). Eighty-four total cases of ovarian serous lesions, including 21 cases of serous cystadenomas, 21 cases of serous borderline tumors, 12 cases of low-grade serous carcinomas and 30 cases of high-grade serous carcinomas were selected from surgical pathology archive of UCLA and Santa Monica medical centers from 2016 to 2019. Among 21 cases of serous borderline tumors, 18 cases were FIGO stage 1 and 3 cases were FIGO stage 2. Also 7 cases were bilateral, 2 cases had micropapillary features, 3 cases had non-invasive implants, 2 cases had progression to low-grade serous carcinoma (including one of the cases with micropapillary features), and 1 case developed supraclavicular and cervical lymph node involvement by serous borderline tumor. Among 12 cases of LGSCs, 1 cases was FIGO stage 1, 1 case was FIGO stage 2 and 10 cases were FIGO stage 3. Ten cases were bilateral, 1 cases was unilateral and 1 case was consistent with peritoneal primary. Ten cases of LGSCs presented with metastasis. The cases of serous cystadenomas and high-grade serous carcinomas were also collected for comparison. The best representative blocks for each case were selected for MTAP immunohistochemistry after reviewing hematoxylin and eosin (H&E).

2.2. Immunostaining of MTAP

Paraffin-embedded sections were cut at 4 μm thickness and paraffin removed with xylene and rehydrated through graded ethanol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 10 min. Heat-induced antigen retrieval (HIER) was carried out for all sections in 0.001 M EDTA buffer, pH = 8.00 using a Biocare decloaker at 95 °C for 25 min. The slides were then incubated for 1 hour at room temperature with rabbit monoclonal MTAP (Abcam, ab126770) at 1/100 dilution. The signal was detected using the rabbit horseradish peroxidase EnVision kit (DAKO, K4003) and visualized with the diaminobenzidine reaction. The sections were counterstained with hematoxylin, air dried and cover slipped.

Cytoplasmic granular staining was used for interpretation of MTAP IHC. Renal tubules of normal kidney tissue were used as positive control tissue for this IHC (Fig. 1). Two pathologists reviewed the stains individually without knowledge about stage and clinical course. Each ovarian serous lesion was labeled as positive (retained) if MTAP showed cytoplasmic staining with the same or greater intensity than internal positive control in more than 50% of the tumor cells and negative (loss of expression) if no cytoplasmic expression or expression at intensity lower than internal positive control observed in more than 50% of the tumor cells, regardless of nuclear staining [12-15].

3. Results

The results of MTAP immunohistochemistry loss is summarized in Table 1. The median age of patients with SBT was 38 years old (range:

Table 1
Summary of all ovarian serous neoplasms with the MTAP loss of expression.

Pathologic diagnosis	Total number of the cases (N = 84)	MTAP loss of expression (%)
Serous cystadenoma	21	0/21 (0%)
Serous borderline tumor	21	10/21 (48%)
Low-grade serous carcinoma	12	8/12 (66%)
High-grade serous carcinoma	30	4/30 (13%)

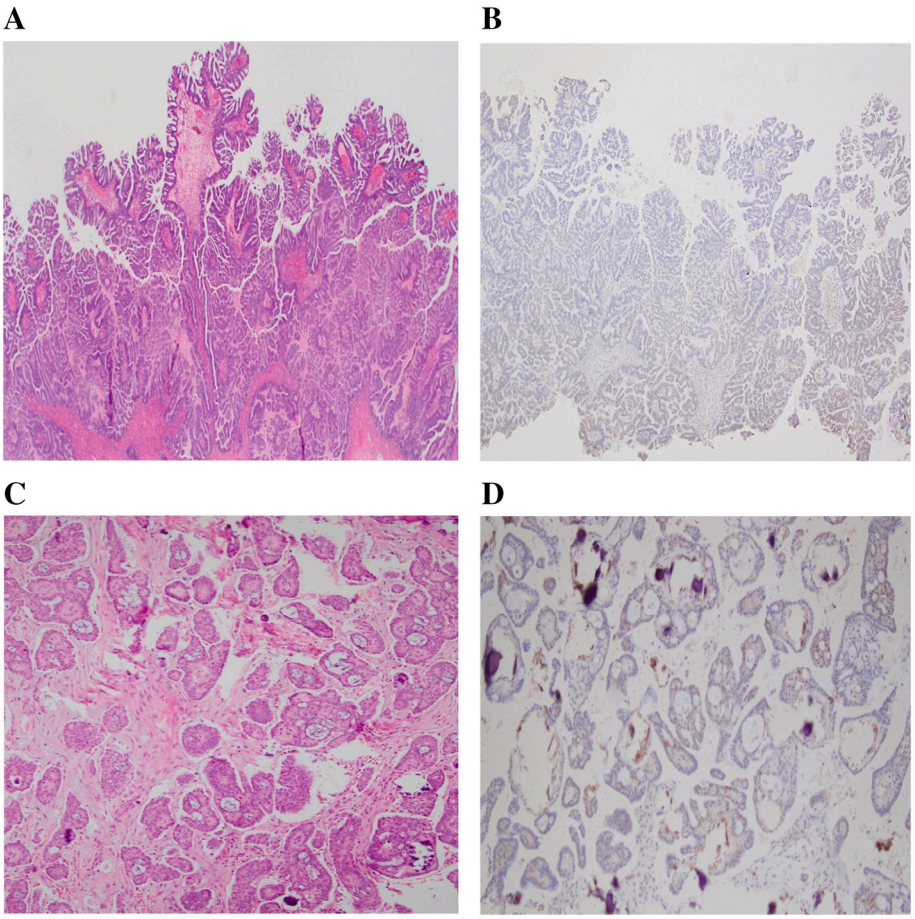

Fig. 2. Ovarian serous borderline tumor, micropapillary variant (H&E) (A). MTAP immunohistochemistry shows loss of expression in the tumor cells (B). Ovarian low-grade serous carcinoma (H&E) (C). MTAP immunohistochemistry shows loss of expression in the carcinoma cells (D).

Table 2
Clinicopathologic features of cases of serous borderline tumor and low grade serous carcinoma with MTAP loss of expression.

Clinicopathologic features	Serous borderline tumor (N = 21)				Low-grade serous carcinoma (N = 12)			
	Overall		MTAP loss of expression		Overall		MTAP loss of expression	
	n	%	n	%	n	%	n	%
Median age	38	Range (16–71)	28	Range (16–71)	68	Range (23–73)	51	Range (23–73)
FIGO stage								
Stage I	18	85%	9/18	50%	1	16%	1/1	100%
Stage II	3	15%	2/3	66%	1	9%	1/1	100%
Stage III–IV	–	–	–	–	10	83%	6/10	60%
Laterality								
Unilateral	14	66%	5/14	35%	1	9%	1/1	100%
Bilateral	7	33%	5/7	71%	10	82%	6/10	60%
Peritoneal	–	–	–	–	1	9%	1/1	100%
Micropapillary features	2	10%	2/2	100%	–	–	–	–
Noninvasive implant	3	14%	2/3	66%	–	–	–	–
Lymph node involvement	1	0.5%	1/1	100%	10	83%	6/10	60%

16–71 years). Among 21 cases, 10 cases showed loss of expression (48%). Five out of 7 bilateral cases, 2 out of 2 cases with micropapillary features and the case with lymph node involvement by serous borderline tumor showed MTAP loss of expression. Also both cases of SBTs with progression to low-grade serous carcinoma showed loss of expression in both SBTs and LGSCs (Fig. 2, A–B). One of these cases was bilateral and interestingly only the right ovarian tumor showed loss of expression. Only 1 of 3 cases of SBTs with non-invasive implant had MTAP loss of expression, and that observed only in the ovarian tumor and not in the implant lesion.

The median age of patients with low-grade serous carcinoma was 68 years old (range: 23–73 years). Eight out of 12 cases of LGSC showed MTAP loss of expression (66%). Among those, one case was status post chemoradiation who presented with disease progression and metastasis and two cases were progressed from SBT within 2 years of initial diagnosis. (Fig. 2, C–D).

Table 2 summarized clinicopathologic features of SBTs and LGSCs with MTAP loss of expression.

Among 30 cases of high-grade serous carcinomas, only 4 cases showed loss of expression for MTAP (13%). Also none of the serous

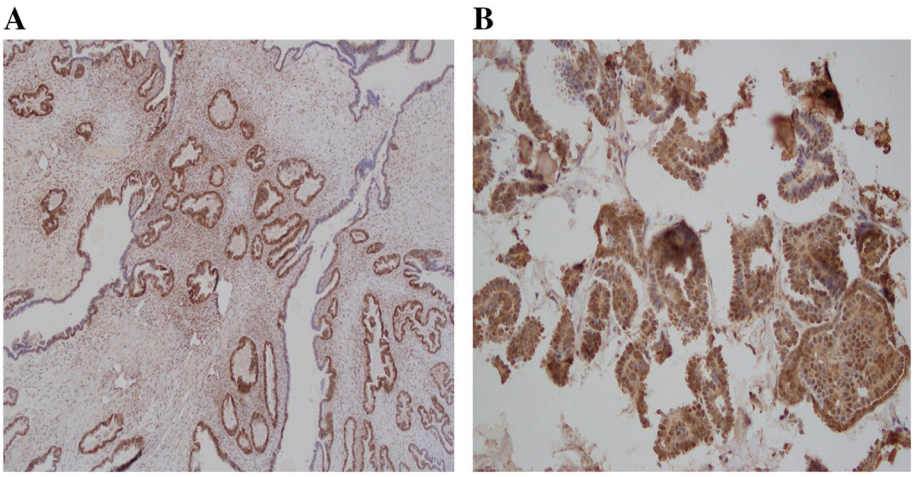

Fig. 3. Cases of ovarian serous borderline tumor (A) and low-grade serous carcinoma (B) with strong expression of MTAP immunohistochemistry in more the 50% of tumor cells.

cystadenoma cases showed loss of expression of MTAP.

Two cases of SBT and LGSC with positive MTAP expression are shown in Fig. 3.

4. Discussion

Although most of serous borderline tumors have excellent clinical course, some features including micropapillary and cribriform morphology and extra-ovarian involvement as a form of invasive implant are associated with adverse clinical behavior [16,17]. Therefore SBTs with micropapillary features are reclassified as "non-invasive low-grade serous carcinoma (niLGSC)" and the tumors with invasive implants renamed as "low-grade serous carcinoma" in 2014 WHO classification [3].

A group at Johns Hopkins in 2004 suggested that serous borderline tumors are arising from serous cystadenoma by demonstrating the same BRAF and KRAS mutations in the lesions with serous cystadenoma adjacent to SBT [18]. Tsang et al. [19] demonstrated the first evidence that LGSCs also may develop from clonal expansion of KRAS mutated clone in serous borderline tumors and also KRAS mutations are commonly detected in recurrent LGSCs. Also studies have suggested that SBTs with BRAF mutation have lower risk of progression to LGSCs [20].

Hunter et al. [6] conducted a study to identify driver genes for predicting clinical behavior of SBTs and LGSCs by using a genome-wide high resolution copy number analysis. Although the overall copy number aberrations (CNA) were higher in LGSCs, they demonstrated that a subset of SBTs also had equal aberration levels with LGSCs, and suggested these may play role in recurrence and progression of SBTs. Among these aberrations, loss of chromosome 9p and homozygous deletions of the CDKN2A locus were significant.

The homozygous deletion of chromosome 9p21which includes the CDKN2A gene, observed in about 15% of the human cancers and usually results in co-deletion of adjacent genes, significantly methylthioadenosine phosphorylase (MTAP) in 80–90% of the cases [21]. MTAP gene, which encodes an important enzyme in methionine salvage pathway, is abundant in normal tissues and has housekeeping function [22]. MTAP has shown to be frequently reduced or lost in many cancer tissues and tumor cell lines [23]. This inactivation most commonly happens due to homozygous deletion of 9p21 region, therefore initially it was presumed that MTAP loss in tumors was due to coincidence. However, now there are evidence that MTAP acts as a tumor suppressor itself [10].

Hida et al. [11] initially suggested MTAP immunohistochemistry for mesothelioma as a highly sensitive and specific surrogate marker for homozygous deletion of chromosome 9p21 locus. Although they used both nuclear and cytoplasmic expression for interpretation, Berg et al. [15] showed that MTAP cytoplasmic staining has more consistency and it is easier to interpret with excellent concordance with CDKN2A deletion by FISH results. Therefore, we also used cytoplasmic staining for interpreting the MTAP IHC in the current study.

Although we had a small sample size, our study showed that there is an association between MTAP loss of expression in SBTs and adverse clinical behavior and likelihood of progression to LGSCs. Seventy-one percent of the bilateral SBTs, both cases with micropapillary features and the SBT with lymph node involvement showed MTAP loss of expression. Also both cases with progression to LGSCs (including one of the micropapillary tumors) had loss of expression in both SBTs and LGSCs. Interestingly, all of the 3 noninvasive implants showed MTAP expression, regardless of expression status of the ovarian tumors. This could be explained by the independent nature of these types of implants, which can have different phenotype from the primary ovarian lesions.

Immunostains like PAX8, epithelial-specific antigen/EpCAM and WT-1 are used for diagnosis in ovarian serous tumors [24]. MTAP is not specific for diagnosis and can be used as a prognostic marker in ovarian serous borderline tumors.

Utilizing a biomarker to identify the tumors with more aggressive behavior, particularly in the early course of the disease is extremely important in management of the patients. To our knowledge, this is the first study to evaluate the MTAP IHC expression in ovarian SBTs and LGSCs. The findings in this study support a role for the investigation of MTAP loss in more aggressive ovarian serous tumors. The significance of loss of MTAP expression is to identify cases with higher risk of future implants, recurrence or progression to low grade serous carcinoma for performing closer follow-up studies on these patients. Future studies in larger series could provide imposing indication of performing this stain as a prognostic marker in these neoplasms.

Funding source

None.

Declaration of competing interest

None.

References

[1] Hannibal CG, Vang R, Junge J, et al. A nationwide study of serous "borderline" ovarian tumors in Denmark 1978–2002: centralized pathology review and overall

survival compared with the general population. Gynecol Oncol 2014;134(2):267–73. https://doi.org/10.1016/j.ygyno.2014.06.002.
[2] Longacre TA, McKenney JK, Tazelaar HD, Kempson RL, Hendrickson MR. Ovarian serous tumors of low malignant potential (borderline tumors): outcome-based study of 276 patients with long-term (> or =5-year) follow-up. Am J Surg Pathol 2005;29(6):707–23. https://doi.org/10.1097/01.pas.0000164030.82810.db.
[3] Hauptmann S, Friedrich K, Redline R, Avril S. Ovarian borderline tumors in the 2014 WHO classification: evolving concepts and diagnostic criteria. Virchows Arch 2017;470(2):125–42. https://doi.org/10.1007/s00428-016-2040-8.
[4] Schmeler KM, Gershenson DM. Low-grade serous ovarian cancer: a unique disease. Curr Oncol Rep 2008;10(6):519–23. https://doi.org/10.1007/s11912-008-0078-8.
[5] Ali RH, Kalloger SE, Santos JL, Swenerton KD, Gilks CB. Stage II to IV low-grade serous carcinoma of the ovary is associated with a poor prognosis: a clinicopathologic study of 32 patients from a population-based tumor registry. Int J Gynecol Pathol 2013;32(6):529–35. https://doi.org/10.1097/PGP.0b013e31827630eb.
[6] Jones S, Wang T-L, Kurman RJ, et al. Low-grade serous carcinomas of the ovary contain very few point mutations. J Pathol 2012;226(3):413–20. https://doi.org/10.1002/path.3967.
[7] Hunter SM, Anglesio MS, Ryland GL, et al. Molecular profiling of low grade serous ovarian tumours identifies novel candidate driver genes. Oncotarget 2015;6(35). https://doi.org/10.18632/oncotarget.5438.
[8] Foulkes WD, Flanders TY, Pollock PM, Hayward NK. The CDKN2A (p16) gene and human cancer. Mol Med 1997;3(1):5–20.
[9] Marjon K, Cameron MJ, Quang P, et al. MTAP deletions in cancer create vulnerability to targeting of the MAT2A/PRMT5/RIOK1 axis. Cell Rep 2016;15(3):574–87. https://doi.org/10.1016/j.celrep.2016.03.043.
[10] Tang B, Kadariya Y, Chen Y, Slifker M, Kruger WD. Expression of MTAP inhibits tumor-related phenotypes in HT1080 cells via a mechanism unrelated to its enzymatic function. G3 2015;5(1):35–44. https://doi.org/10.1534/g3.114.014555.
[11] Hida T, Hamasaki M, Matsumoto S, et al. Immunohistochemical detection of MTAP and BAP1 protein loss for mesothelioma diagnosis: comparison with 9p21 FISH and BAP1 immunohistochemistry. Lung Cancer 2017;104:98–105. https://doi.org/10.1016/j.lungcan.2016.12.017.
[12] Kinoshita Y, Hida T, Hamasaki M, et al. A combination of MTAP and BAP1 immunohistochemistry in pleural effusion cytology for the diagnosis of mesothelioma: MTAP and BAP1 for mesothelioma diagnosis. Cancer Cytopathol 2018;126(1):54–63. https://doi.org/10.1002/cncy.21928.
[13] Krasinskas AM, Bartlett DL, Cieply K, Dacic S. CDKN2A and MTAP deletions in peritoneal mesotheliomas are correlated with loss of p16 protein expression and poor survival. Mod Pathol 2010;23(4):531–8. https://doi.org/10.1038/modpathol.2009.186.
[14] Chapel DB, Schulte JJ, Berg K, et al. MTAP immunohistochemistry is an accurate and reproducible surrogate for CDKN2A fluorescence in situ hybridization in diagnosis of malignant pleural mesothelioma. Mod Pathol 2020;33(2):245–54. https://doi.org/10.1038/s41379-019-0310-0.
[15] Berg KB, Dacic S, Miller C, Cheung S, Churg A. Utility of methylthioadenosine phosphorylase compared with BAP1 immunohistochemistry, and CDKN2A and NF2 fluorescence in situ hybridization in separating reactive mesothelial proliferations from epithelioid malignant mesotheliomas. Arch Pathol Lab Med 2018;142(12):1549–53. https://doi.org/10.5858/arpa.2018-0273-OA.
[16] Burks RT, Sherman ME, Kurman RJ. Micropapillary serous carcinoma of the ovary. A distinctive low-grade carcinoma related to serous borderline tumors. Am J Surg Pathol 1996;20(11):1319–30. https://doi.org/10.1097/00000478-199611000-00003.
[17] Seidman JD, Kurman RJ. Subclassification of serous borderline tumors of the ovary into benign and malignant types. A clinicopathologic study of 65 advanced stage cases. Am J Surg Pathol 1996;20(11):1331–45. https://doi.org/10.1097/00000478-199611000-00004.
[18] Ho C-L, Kurman RJ, Dehari R, Wang T-L, Shih I-M. Mutations of BRAF and KRAS precede the development of ovarian serous borderline tumors. Cancer Res 2004;64(19):6915–8. https://doi.org/10.1158/0008-5472.CAN-04-2067.
[19] Tsang YT, Deavers MT, Sun CC, et al. KRAS (but not BRAF) mutations in ovarian serous borderline tumour are associated with recurrent low-grade serous carcinoma. J Pathol 2013;231(4):449–56. https://doi.org/10.1002/path.4252.
[20] Chui MH, Kjaer SK, Frederiksen K, et al. BRAFV600E-mutated ovarian serous borderline tumors are at relatively low risk for progression to serous carcinoma. Oncotarget 2019;10(64):6870–8. https://doi.org/10.18632/oncotarget.27326.
[21] Zhang H, Chen Z-H, Savarese TM. Codeletion of the genes for p16INK4, methylthioadenosine phosphorylase, interferon-α1, interferon-β1, and other 9p21 markers in human malignant cell lines. Cancer Genet Cytogenet 1996;86(1):22–8. https://doi.org/10.1016/0165-4608(95)00157-3.
[22] Zappia V, Ragione FD, Pontoni G, Gragnaniello V, Cartenì-Farina M. Human 5′-deoxy-5′-methylthioadenosine phosphorylase: kinetic studies and catalytic mechanism. In: Zappia V, Pegg AE, editors. Progress in polyamine research: novel biochemical, pharmacological, and clinical aspects. Advances in experimental medicine and biology Springer US; 1988. p. 165–77. https://doi.org/10.1007/978-1-4684-5637-0_15.
[23] Ding N, Yin F, Deng X, et al. MTAP deficiency is associated with an unfavourable prognosis and platinum resistance in ovarian cancer. Int J Clin Exp Pathol 2017;10(2):1122–30.
[24] Kaspar HG, Crum CP. The utility of immunohistochemistry in the differential diagnosis of gynecologic disorders. Arch Pathol Lab Med 2015;139:39–54.