DNA mismatch repair deficiency and p53 abnormality are age-related events in mixed endometrial carcinoma with a clear cell component

Naoyuki Ida 1, Keiichiro Nakamura 1, Masayuki Saijo 2, Atsuko Nasu 3, Tadashi Yoshino 4, Hisashi Masuyama 1, Hiroyuki Yanai, MD 3,
1 Department of Obstetrics and Gynecology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan
2 Department of Obstetrics and Gynecology, Himeji Red Cross Hospital, Himeji, Japan
3 Department of Pathology, Okayama University Hospital, Okayama, Japan
4 Department of Pathology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan
Address for correspondence and reprint requests to Hiroyuki Yanai
2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan
E-mail: yanaih@md.okayama-u.ac.jp
Phone: +81-86-235-7754
Fax: +81-86-235-7756

Declarations

Conflicts of interests: none declared.

Ethics approval: This study was approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences and Okayama University Hospital (1704-022).

Abstract

Mixed endometrial carcinoma (MEC) is defined as a tumor composed of two or more spatially distinct subtypes, at least one of which is serous or clear cell carcinoma. In this study, the clinicopathological features of 15 MEC cases containing a clear cell component (MEC-C) were investigated. The ages of patients ranged from 32 to 83 years (median, 61 years). The combinations of carcinoma components observed were endometrioid and clear cell in ten patients; endometrioid, clear cell and serous in three; and clear cell and serous in two. Immunohistochemically, nine had DNA mismatch repair (MMR) protein deficiency (MMR-d), nine had loss of ARID1A and three cases had aberrant p53 expression. MMR-d and loss of ARID1A showed a strong correlation. Only one case showed both MMR-d and aberrant p53 expression. The patients with MMR-d were younger than those without MMR-d (median; 58 years vs. 71 years). Loss of ARID1A also showed significant predilection for younger women than ARID1A intact cases. In conclusion, MMR-d was observed in 60% of MEC-C, showed predilection for young

women, and was associated with ARID1A loss. In contrast, non- MMR-d MEC-C occurred in elder women and some tumors may associate with TP53 mutation. These findings suggest that MEC-C develop via two different molecular mechanisms and they are age-related events.

Keywords: Mixed endometrial carcinoma, clear cell carcinoma, endometrium, p53, mismatch repair, ARID1A

Introduction

Mixed endometrial carcinoma (MEC) is defined as a carcinoma composed of two or more spatially distinct tumor subtypes, and at least one of which is a serous or a clear cell carcinoma. The prognosis of endometrial carcinoma with even a minor serous or clear cell components is poorer than that of pure low-grade endometrioid carcinoma, hence any amount of serous or clear cell carcinoma component warrants a diagnosis of MEC [1, 2]. MEC is rare, accounting for 2.5% of all malignant epithelial tumors of the endometrium in Japan [3]. According to a large-scale study of MEC, 40-50% of MECs contain clear cell components [4].

Endometrial clear cell carcinoma (ECCC) is a rare endometrial carcinoma with characteristic histological features such as clear or oxyphilic cytoplasm, hobnail cells, and small round papillary structures often associated with a hyalinized stroma. However, these histological features sometimes overlap with endometrioid or serous carcinomas [5, 6]. Molecular subclassification according to The Cancer Genome Atlas (TCGA) scheme identifies that ECCC as a heterogeneous group of tumors [7-11]. These morphological and molecular overlaps cause poor diagnostic reproducibility of ECCC even among specialized gynecologic pathologists [12-14].

In this study, we focused on MEC containing an ECCC component (MEC-C), and performed clinicopathological analysis to clarify the molecular features and biology of MEC-C.

Materials and Methods

Patients

This study was approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences and Okayama University Hospital (1704-022). In this retrospective study, the pathology files of Okayama University Hospital between January 2006 and December 2019 were reviewed. Among 605 hysterectomy cases of endometrial carcinoma, 18 were recorded as MEC-C or

carcinoma containing an ECCC component. After a histological review of these cases, 3 were excluded because 1 was considered as a pure serous carcinoma and 2 were considered as a pure clear cell carcinoma. The remaining 15 cases (2.5%) were included in this study.

All patients underwent a total hysterectomy, bilateral salpingo-oophorectomy with or without omentectomy, and pelvic and/or para-aortic lymphadenectomy. In all patients, the lymph node status was assessed using computed tomography (CT) with or without positron emission tomography (PET). Lymph nodes with short-axis lengths >10.0 mm on PET-CT or CT were defined as metastatic. No patients received neoadjuvant treatment.

Histopathological review

Histological slides from all cases were reviewed by the authors (NI, MS and HY), who were blinded to the clinical course and IHC status data. The histological findings listed in the diagnostic recommendation of the International Society of Gynecological Pathologists for clear cell components were recorded [15]. Histological findings such as structure (usual or small round papillary structure without diffuse stratification, tubulocystic and/or solid configuration), and cytologic features (cuboidal, polygonal, or hobnail cells with clear or eosinophilic cytoplasm) were evaluated. Carcinoma mimicking clear cell carcinoma such as endometrioid carcinoma of secretory variant or nonspecific clear cells, and serous carcinoma were carefully excluded. Additionally, existence of microcystic, elongated, and fragmented glands (MELF) [16], lymphovascular space invasion (LVSI), squamous and/or mucinous differentiation were evaluated. The number of tumor-infiltrating lymphocytes (TIL) was evaluated as described by Shia et al [17], and cases with more than 40 TIL per 10 high-power fields (total area, 2.37 mm2) were evaluated as TIL positive.

Immunohistochemistry

To investigate tumorigenesis, immunohistochemistry (IHC) for p53, p16, DNA mismatch repair (MMR) proteins (MLH1, MSH2, MSH6 and PMS2), ARID1A, and PTEN was performed. The immunohistochemical profile of ECCC was examined using several markers, including the estrogen receptor (ER), progesterone receptor (PgR), HNF-1β, and Napsin A.

The primary antibodies used in the study are listed in Table 1. Slides from representative formalin-fixed paraffin-embedded blocks were stained using an automated staining device (Benchmark Ultra; Ventana Medical Systems, Tucson, AZ), and the ultraView or OptiView staining kit (Ventana Medical Systems). Expression of p53 and MMR proteins was evaluated as previously described [18], and tumors lacking

expression of any of the MMR proteins were classified as MMR-d. Strong, diffuse nuclear and cytoplasmic p16 staining, was considered as positive. The absence of PTEN and ARID1A staining, with positive staining of adjacent stromal cells, was considered loss of expression. Napsin A immunostaining was considered positive when more than 5% of the tumor cells exhibited cytoplasmic staining. ER, PgR, and HNF-1ß were considered positive if the tumor exhibited at least moderate nuclear staining intensity in >70% of tumor cells.

Results

The clinicopathological characteristics of patients with MEC-C are summarized in Table 2. All patients were Japanese and patient age at diagnosis ranged from 32 to 83 years (median, 61 years), with 80% of the patients diagnosed with FIGO (International Federation of Gynecology and Obstetrics) stage I. Histologically, ten tumors had endometrioid and clear cell components; three tumors had endometrioid, clear cell and serous components; and two tumors had clear cell and serous components.

Representative histologic images of MEC-C are shown in Fig. 1. In seven (46.7%) cases, the clear cell component occupied less than 5%. The most common architecture was a papillary structure (13/15, 86.7%), followed by solid structure (10/15, 66.7%). A combination of papillary and solid configuration was seen in seven (46.7%) tumors, and small round papillae were observed in seven (46.7%) tumors. Other features such as hobnail cells (8/15, 53.4%), hyaline stroma (6/15, 40.0%), and hyaline globules (4/15, 26.7%) were also observed.

MELF were seen in one case of mixed endometrioid and clear cell carcinoma. Seven cases showed LVSI and three of these patients had lymph node metastasis. Squamous differentiation and mucinous differentiation were seen in one case. Six cases were TIL positive.

The immunohistochemistry results are summarized in Table 3 and representative immunohistochemistry images are shown in Fig. 2 and 3. Aberrant expression of p53 was identified in three (20.0%) cases, two of which had serous components. In all cases, the IHC status of p53 was identical in both clear cell and non-clear cell components. The ages of the three patients with aberrant p53 expression were 60, 68, and 80.

Two cases showed diffuse expression of p16 in both clear cell and non-clear cell components. One of them showed p53 aberrant expression. None of the MMR-d cases showed p16 diffuse expression.

MMR-d was identified in nine cases (60.0%). In all cases, the clear cell and non-clear cell component showed identical MMR protein expression. LVSI was seen in five of the nine

MMR-d cases (55.6%) and two of the six non-MMR-d cases (33.3%). TIL positive were more frequent in MMR-d cases than in non-MMR-d cases (55.6% versus 16.7%). One tumor (case 7) showed concurrent overexpression of p53 and loss of MLH1 expression (Fig. 3). The median age of patients with MMR-d MEC-C was 58 years (range, 32-72 years), and with non-MMR-d MEC-C was 71 years (range; 61-83 years). The morphological characteristics of clear cell components between MMR-d and non-MMR-d cases were compared, and no differences were observed.

ARID1A loss was observed in nine (60.0%) tumors in both clear and non-clear components. Among MMR-d cases, eight of nine cases lacked ARID1A expression (88.9%). In contrast, only one of six cases (16.7%) of non-MMR-d cases lacked ARID1A.

Loss of PTEN could be evaluated in thirteen cases and it was observed in eight cases (61.5%). In these cases, both of clear cell and non-clear cell component were negative for PTEN. One case was morphologically mixed serous and clear cell carcinoma, but it showed a wild type p53 pattern and MMR-d.

ER and PgR could be evaluated in fourteen cases. Clear cell component was positive for ER in one case (7.1%) and PgR was negative in all cases. In contrast, non-clear cell component was positive for ER and PgR in seven and two cases, respectively (50.0% and 14.3%).

HNF-1β expression in clear cell components was found in all tumors. Although HNF-1β staining was observed in both clear cell and non-clear cell components, clear cell components tended to show stronger HNF-1β expression than non-clear cell components.

Napsin A expression was positive in eight (53.3%) tumors and was observed only in the clear cell component.

Discussion

The prognosis of MEC is poorer than that of pure endometrioid carcinoma, hence accurate diagnosis is important. The key to MEC diagnosis is the recognition of serous or clear cell component. However, ECCC is rare, and some non-clear cell endometrial carcinomas can have a clear cytoplasm, as a result of this and the heterogeneity of ECCC, the reproducibility of ECCC diagnosis is modest [14]. Tumor cells of the secretory variant of endometrioid carcinoma have sub- or supranuclear vacuoles in the cytoplasm and mimic ECCC. In addition to the secretory variant, nonspecific clear cytoplasm can be seen in a minor proportion of endometrioid carcinomas [5]. Usually, these carcinomas lack the histological features of ECCC, such as small, round papillary configuration, tubulocystic pattern, hyalinized stroma, and nuclear pleomorphism. Serous carcinomas can show clear cell proliferation, and share high-grade nuclear atypia

and hobnail-shaped cells with ECCC as well. Previous studies indicated that 14-32% of serous carcinomas contain “clear cell” components [6, 19].

These diagnostic complications of ECCC result in low rates of MEC-C diagnosis reproducibility [20]. Besides these diagnostic problems, the prognosis of MEC-C is poorer than that of pure endometrioid carcinoma [1], but better than that of pure ECCC [21]. In this study, we adopted the morphological criteria recommended by the International Society of Gynecologic Pathologists [15]. In addition, we used supportive immunohistochemical positive markers such as HNF-1β and Napsin A, with all cases expressing at least one of these markers. Moreover, clear cell component in our series of MEC-C less frequently expressed ER and PgR than non-clear cell component. This finding is consistent with the rare expression of ER and PgR in ECCC [22-24].

Recent studies revealed that MEC-C frequently shows MMR-d and that both clear and non-clear components harbor common genetic changes. The latter findings can indicate derivation of both components from a single clone [25]. Here, we performed an immunohistochemical study and found a relatively high frequency of MMR-d, loss of ARID1A, and low frequency of aberrant p53 expression. The clear cell and non-clear cell components demonstrate identical immunohistochemical findings of these molecules, and these results were consistent with previous observations.

MMR-d is a characteristic molecular finding in endometrial carcinoma. In previous studies, the frequencies of MMR-d or microsatellite instability in ECCC were inconsistent and ranged from 0 to 68.8% [8-11, 24, 26]. A previous study of MEC-C demonstrated loss of at least one of the MMR proteins expression in 66% of these tumors [21]. The results of our study are consistent with this observation, as 60% of the tumors show the MMR-d phenotype. A recent study of MEC composed of endometrioid and serous carcinoma in patients younger than 60 years, showed a high frequency of MMR-d (5 of 12, 41.7%), which is uncommon in pure serous carcinoma [27]. This result and our findings in MEC-C suggest that MMR-d may cause genetic instability, and hence induces morphologic plasticity in endometrial carcinomas in young women.

MMR-d endometrial carcinomas show characteristic histological findings. In this study, we found that LVSI and TIL were more frequently seen in MMR-d cases. Some previous reports showed an increased frequency of LVSI and a higher number of TIL in MMR-d endometrial carcinoma [17, 28-33]. We also observed these trends in MEC-C and suggest that histological features could be clue for MMR-d cases.

Mutation or loss of immunoreactivity of ARID1A has been detected in 8-25% of pure ECCC cases [10, 26, 34, 35] and in three of five MEC-C cases [25]. In our study, ARID1A loss was found in nine of fifteen cases, eight of which showed concurrent MMR-d. Only

one case with ARID1A loss showed aberrant expression of p53. The relationship between ARID1A and p53 expression is controversial. One study indicated that ARID1A loss was not present in p53 wild-type pure ECCC [11]. However, in other studies, ARID1A loss correlated with the expression of wild type p53 [10, 36]. It has been suggested that ARID1A may cause microsatellite instability, by contributing to the epigenetic silencing of the MLH1 gene in endometrial cancer [37]. In the present study, seven of nine cases with ARID1A loss also lost MLH1 expression, supporting this hypothesis. Our results suggest that, loss of ARID1A function results in MMR-d phenotype and induces clear cell morphology.

Previous studies have demonstrated aberrant expression of p53 in up to one-third of ECCC [10, 24, 38]. Aberrant p53 expression was previously observed in one of five cases of mixed endometrioid and clear cell carcinoma, and one of two mixed serous and clear cell carcinoma [25]. In our study, aberrant expression of p53 was observed in three of fifteen MEC-C and all patients were older than 60 years. Among them, two tumors contained serous carcinoma components. Aberrant p53 expression was observed in only one of nine tumors with MMR-d, and similar results were reported in a previous study [25]. These findings suggest that MMR-d and p53 abnormalities are independent molecular mechanisms of tumorigenesis in most MEC-C.

PTEN mutations are one of the most common molecular abnormalities of endometrioid carcinoma and detected in more than 80% ECCC. Frequency of PTEN mutations differ from 0 to 21% [7, 9, 10, 39]. One report showed loss of PTEN in 81.3% of ECCC [26]. High frequency of PTEN loss in our study showed that MEC-C is more similar to endometrioid carcinoma than pure ECCC. Here, we found that eight of nine MEC-C with PTEN loss had an endometrioid component. It is suggested that these tumors occurred as endometrioid carcinoma and the clear cell component developed later.

HNF-1β is a highly sensitive and specific marker of ovarian clear cell carcinoma; however, it is less sensitive and specific in ECCC [20]. HNF-1β expression is seen in 83-100% of ECCC cases [34, 40]. In our study, all cases showed immunoreactivity for HNF-1β in clear cell components. However, in eight cases (53.3%), both clear cell and non-clear cell components showed the same HNF-1β intensity. Therefore, while HNF-1β was associated with clear-cell morphology, the finding requires careful interpretation.

Napsin A is a diagnostic marker for ovarian clear cell carcinoma and is positive in 15-75% of ECCC or MEC-C [21, 34, 41-44]. In the present study, seven of fifteen (46.7%) clear cell components showed Napsin A staining, but the non-clear cell components were negative. The role of Napsin A in the development, pathogenesis, and progression of ECCC is unclear; however, it may help distinguish clear cell carcinoma from other

histologies.

The main limitation of our study is the small sample size, and that we could not conclude a relationship between prognosis and clinicopathological features. A previous study demonstrated better prognosis in patients with MEC-C than those with pure ECCC, and among MEC-C patients, the prognosis in MMR-d patients was favorable [21]. In our study, all MEC-C patients with MMR-d are alive but two patients with intact MMR died due to disease.

In conclusion, MMR-d was observed in 60% of MEC-C, showed a predilection for young women, and association with ARID1A loss. In contrast, non- MMR-d MEC-C was observed in elder women, and some tumors may associate with TP53 mutation. These findings suggest that MEC-C develop via two different molecular mechanisms, which are age-related events. In view of diverse prognosis and possible effects of immune checkpoint reagents [45], MMR status should be examined at the time of MEC-C diagnosis, and the results should be taken into consideration in decision making for patient management.

Acknowledgement

We would like to thank Editage for editing and reviewing this manuscript for English language.

Table 1. Primary antibodies used in this study.

Antigen	Source	Clone	Detection	Dilution
p53	Agilent	DO7	ultraView	Prediluted
p16	SantaCruz	JC8	ultraView	1:500
ARID1A	Sigma-Aldrich	Polyclonal	ultraView	1:100
MLH1	Roche	M1	OptiView	Prediluted
PMS2	Roche	A16-4	OptiView	Prediluted
MSH2	Roche	G219-1129	OptiView	Prediluted
MSH6	Roche	SP93	OptiView	Prediluted
Napsin A	Nichirei	Polyclonal	ultraView	Prediluted
HNF-1β	Sigma-Aldrich	Polyclonal	ultraView	1:100
PTEN	Roche	SP218	ultraView	Prediluted
ER	Roche	SP1	ultraView	Prediluted
PgR	Roche	1E2	ultraView	Prediluted

ER: estrogen receptor, PgR: progesterone receptor. Agilent (Santa Clara, CA), Santa Cruz (Dallas, TX), Sigma-Aldrich (St. Louis, MO), Roche (Tucson, AZ), Nichirei (Tokyo, Japan).

Table 2. Clinicopathological features.

No.	Age	Histology	FIGO Stage	Mucinous or squamous	LVSI	TIL	MELF	Follow-up(months)	
1	32	EmG1/CC	IIIC1	(–)	(+)	(+)	(–)	86	NED
2	52	EmG2/CC	IB	(–)	(+)	(+)	(–)	157	NED
3	53	EmG2/CC	IA	Muc	(+)	(+)	(–)	23	NED
4	57	EmG1/CC	IA	(–)	(+)	(–)	(–)	155	NED
5	58	EmG1/CC	IA	(–)	(–)	(+)	(–)	1	NED
6	58	SC/CC	IIIC1	(–)	(+)	(–)	(–)	18	AWD
7	60	EmG1/CC	IA	(–)	(–)	(–)	(–)	41	AWD
8	61	EmG1/CC	IA	(–)	(–)	(–)	(–)	109	NED
9	62	EmG3/CC	IIIC1	(–)	(+)	(–)	(–)	28	NED
10	66	EmG1/CC	IA	(–)	(–)	(–)	(+)	16	NED
11	68	SC/CC	IA	(–)	(–)	(–)	(–)	65	DOOD
12	71	EmG1CC/SC	IA	Sq	(–)	(+)	(–)	47	DOD
13	72	EmG1/CC	IA	(–)	(–)	(–)	(–)	5	NED
14	80	EmG3/CC/SC	IA	(–)	(–)	(–)	(–)	61	NED
15	82	EmG1/CC	IA	(–)	(+)	(+)	(–)	10	DOD

CC: clear cell carcinoma, DOD: died of disease, DOOD: died of other disease, Em: endometrioid, FIGO: International Federation of Gynecology and Obstetrics, G: grade, LVSI: lymphovascular space invasion, MELF: microcystic, elongated, and fragmented glands, NED: no evidence of disease, SC: serous carcinoma, TIL: tumor infiltrating lymphocytes.

Table 3. Immunohistochemical findings.

No.	p53	p16	MMR-d (loss)	ARID1A	PTEN	HNF-1β non-CC/CC	NapsinA	ER non-CC/CC	PgR non-CC/CC
1	wt	(–)	MLH1, PMS2	(–)	(–)	(–)/(+)	(–)	(–)/(–)	(–)/(–)
2	wt	(–)	MLH1, PMS2	(–)	NA	(–)/(–)	(–)	NA	NA
3	wt	(–)	MSH2, MSH6	(–)	NA	(–) (+)	(–)	(+)/(–)	(–)/(–)
4	wt	(–)	MLH1, PMS2, MSH6	(–)	(–)	(–) /(+)	(–)	(+)/(–)	(–)/(–)
5	wt	(–)	PMS2	(+)	(–)	(–)/(+)	(+)	(+)/(–)	(+)/(–)
6	wt	(–)	MLH1, PMS2	(–)	(–)	(+)/(+)	(–)	(–)/(–)	(–)/(–)
7	Aberrant	(–)	MLH1, PMS2	(–)	(–)	(+)/(+)	(+)	(+)/(–)	(–)/(–)
8	wt	(–)	(–)	(+)	(+)	(+)/(+)	(+)	(–)/(–)	(–)/(–)
9	wt	(–)	(–)	(–)	(–)	(–)/(+)	(+)	(–)/(–)	(–)/(–)
10	wt	(–)	MLH1, PMS2	(–)	(+)	(+)/(+)	(+)	(+)/(+)	(–)/(–)
11	Aberrant (null)	(–)	(–)	(+)	(+)	(+)/(+)	(+)	(–)/(–)	(–)/(–)
12	wt	(–)	(–)	(+)	(–)	(+)/(+)	(–)	(–)/(–)	(–)/(–)
13	wt	(–)	MLH1, PMS2	(–)	(–)	(–)/(+)	(–)	(–)/(–)	(–)/(–)
14	Aberrant	(+)	(–)	(+)	(+)	(+)/(+)	(+)	(+)/(–)	(–)/(–)
15	wt	(+)	(–)	(+)	(–)	(–) (+)	(+)	(+)/(–)	(+)/(–)

CC: clear cell, NA: not available, wt: wild type.

Figure Legends

Fig 1 Histological appearance of the clear cell component. (a) Solid growth of tumor cells with clear cytoplasm. Nuclei showed high-grade atypia. (b) Small round papillary growth with myxoid stroma. (c) Hyalinized stroma. (d) Hobnail cells.

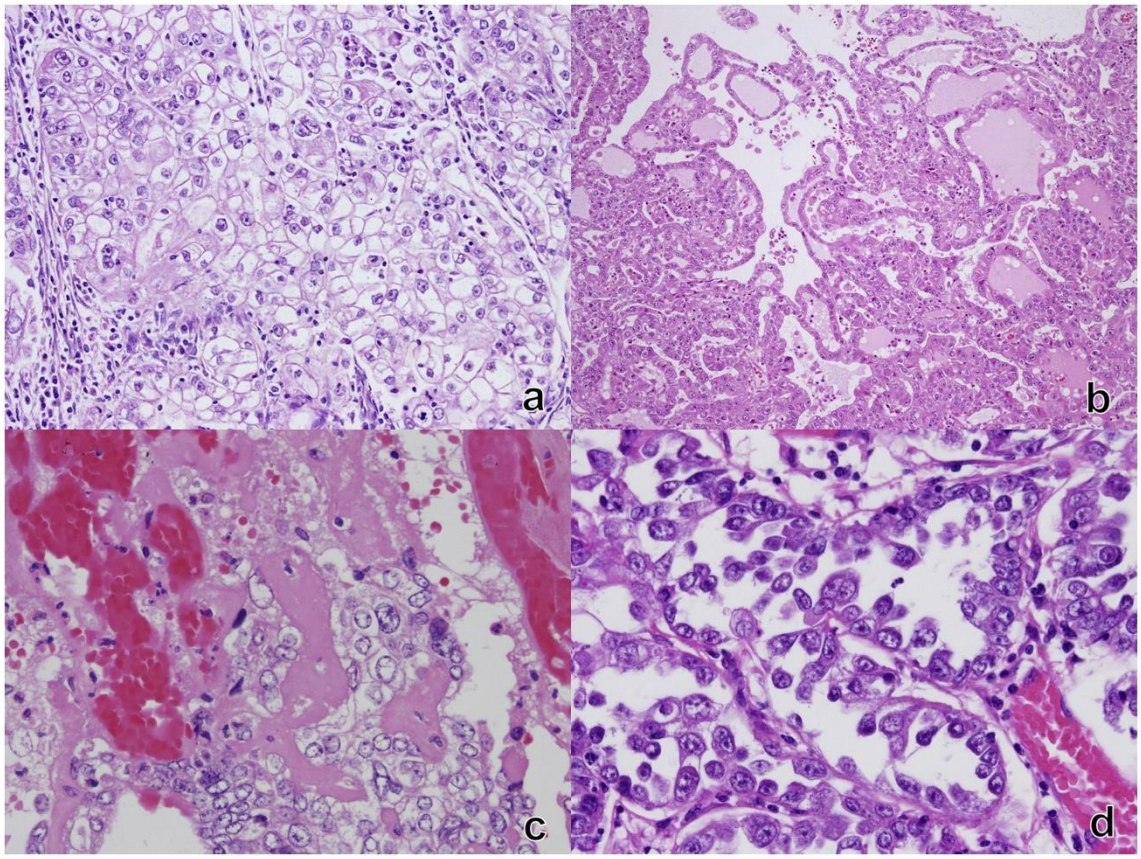


Fig 2 Histology and immunohistochemistry of Case 5. (a) Clear cell component (left, lower) and endometrioid component (right). (b) Clear cell component showed papillary proliferation of hobnail cells. Clear cells were positive for HNF-1β(c) and Napsin A (d).

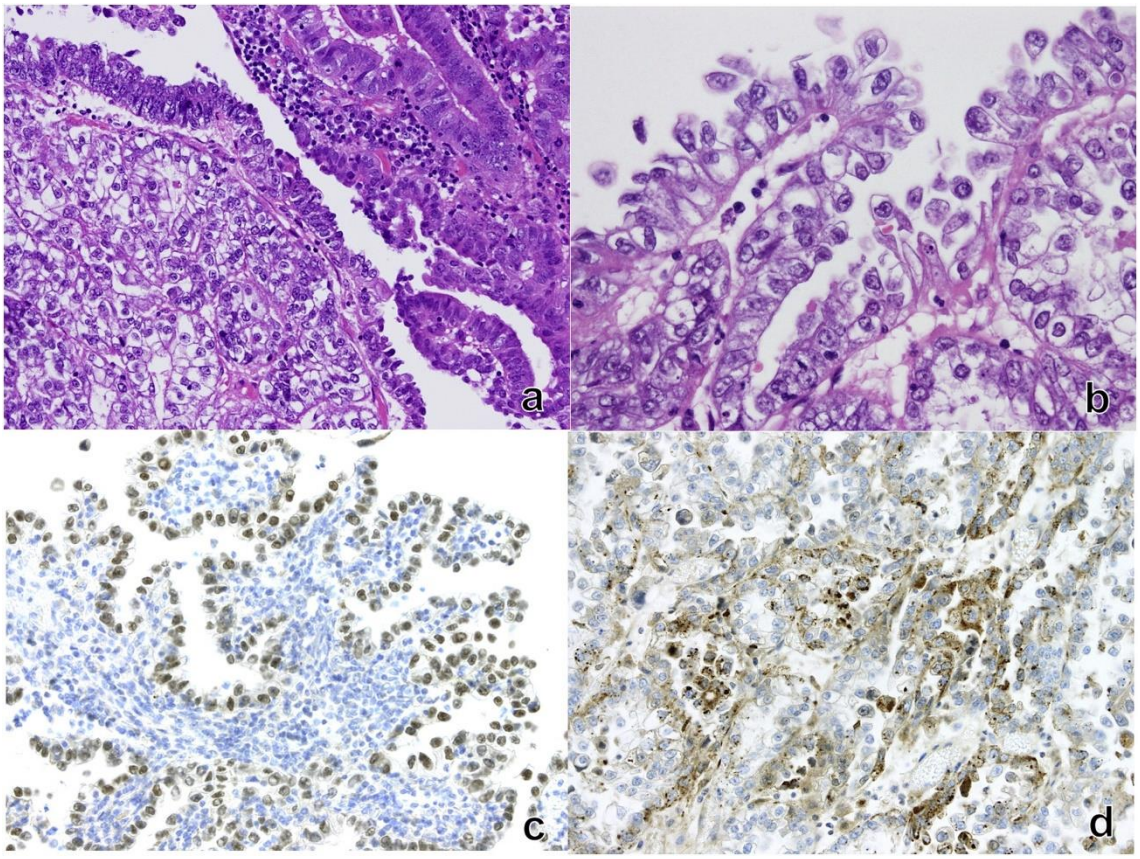
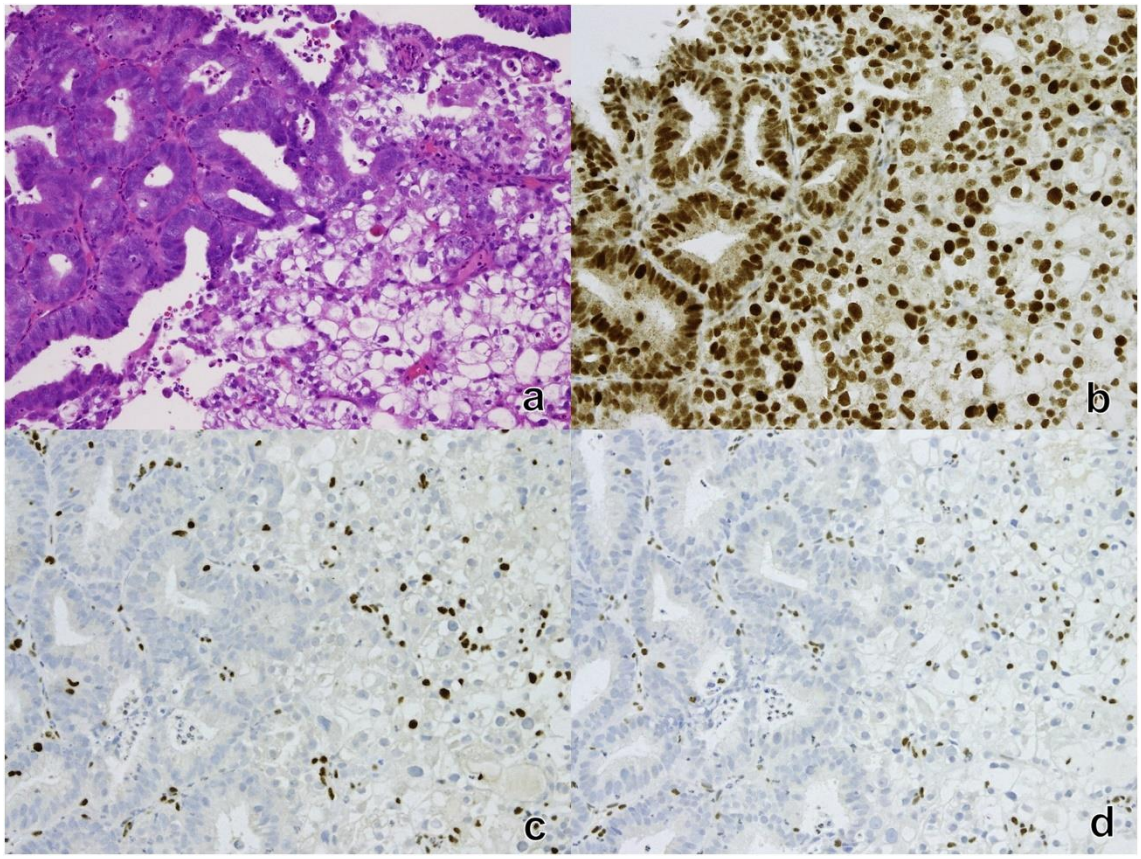


Fig 3 Histology and immunohistochemistry of Case 7. (a) Clear cell component (right) and endometrioid component (left). Both the endometrioid (left) and clear cell (right) components showed aberrant expression of p53 (b) loss of MLH1 expression (c) and ARID1A (d).



[1] M.R. Quddus, C.J. Sung, C. Zhang, W.D. Lawrence, Minor serous and clear cell components adversely affect prognosis in "mixed-type" endometrial carcinomas: a clinicopathologic study of 36 stage-I cases. Reprod Sci 17 (2010) 673-678. 10.1177/1933719110368433

[2] J.T. Rabban, C.B. Gilks, A. Malpica, X. Matias-Guiu, K. Mittal, G.L. Mutter, E. Oliva, V. Parkash, B.M. Ronnett, P. Staats, C.J.R. Stewart, W.G. McCluggage, Issues in the Differential Diagnosis of Uterine Low-grade Endometrioid Carcinoma, Including Mixed Endometrial Carcinomas: Recommendations from the International Society of Gynecological Pathologists. Int J Gynecol Pathol 38 Suppl 1 (2019) S25-s39. 10.1097/pgp.0000000000000512

[3] S. Nagase, T. Ohta, F. Takahashi, T. Enomoto, Annual report of the committee on gynecologic oncology, the Japan Society of Obstetrics and Gynecology: Annual patients report for 2015 and annual treatment report for 2010. J Obstet Gynaecol Res 45 (2019) 289-298. 10.1111/jog.13863

[4] W. Li, L. Li, M. Wu, J. Lang, Y. Bi, The Prognosis of Stage IA Mixed Endometrial Carcinoma. Am J Clin Pathol 152 (2019) 616-624. 10.1093/ajcp/aqz083

[5] E.G. Silva, R.H. Young, Endometrioid neoplasms with clear cells: a report of 21 cases in which the alteration is not of typical secretory type. Am J Surg Pathol 31 (2007) 1203-1208. 10.1097/PAS.0b013e3180339ed7

[6] N. Hariri, M. Qarmali, O. Fadare, Endometrial Serous Carcinoma With Clear-Cell Change: Frequency and Immunohistochemical Analysis. Int J Surg Pathol 26 (2018) 126-134. 10.1177/1066896917731862

[7] L.N. Hoang, M.K. McConechy, B. Meng, J.B. McIntyre, C. Ewanowich, C.B. Gilks, D.G. Huntsman, M. Kobel, C.H. Lee, Targeted mutation analysis of endometrial clear cell carcinoma. Histopathology 66 (2015) 664-674. 10.1111/his.12581

[8] E. Stelloo, T. Bosse, R.A. Nout, H.J. MacKay, D.N. Church, H.W. Nijman, A. Leary, R.J. Edmondson, M.E. Powell, E.J. Crosbie, H.C. Kitchener, L. Mileshkin, P.M. Pollock, V.T. Smit, C.L. Creutzberg, Refining prognosis and identifying targetable pathways for high-risk endometrial cancer; a TransPORTEC initiative. Mod Pathol 28 (2015) 836-844. 10.1038/modpathol.2015.43

[9] M. Le Gallo, M.L. Rudd, M.E. Urick, N.F. Hansen, S. Zhang, F. Lozy, D.C. Sgroi, A. Vidal Bel, X. Matias-Guiu, R.R. Broaddus, K.H. Lu, D.A. Levine, D.G. Mutch, P.J. Goodfellow, H.B. Salvesen, J.C. Mullikin, D.W. Bell, Somatic mutation profiles of clear cell endometrial tumors revealed by whole exome and targeted gene sequencing. Cancer 123 (2017) 3261-3268. 10.1002/cncr.30745

[10] D.F. DeLair, K.A. Burke, P. Selenica, R.S. Lim, S.N. Scott, S. Middha, A.S. Mohanty,

D.T. Cheng, M.F. Berger, R.A. Soslow, B. Weigelt, The genetic landscape of endometrial clear cell carcinomas. J Pathol 243 (2017) 230-241. 10.1002/path.4947
[11] N. Baniak, O. Fadare, M. Köbel, J. DeCoteau, V. Parkash, J.L. Hecht, K.Z. Hanley, K. Gwin, W. Zheng, C.M. Quick, E.A. Jarboe, S.X. Liang, M. Kinloch, Targeted Molecular and Immunohistochemical Analyses of Endometrial Clear Cell Carcinoma Show that POLE Mutations and DNA Mismatch Repair Protein Deficiencies Are Uncommon. Am J Surg Pathol 43 (2019) 531-537. 10.1097/pas.0000000000001209
[12] O. Fadare, V. Parkash, W.D. Dupont, G. Acs, K.A. Atkins, J.A. Irving, E.C. Pirog, B.J. Quade, M.R. Quddus, J.T. Rabban, 3rd, R. Vang, J.L. Hecht, The diagnosis of endometrial carcinomas with clear cells by gynecologic pathologists: an assessment of interobserver variability and associated morphologic features. Am J Surg Pathol 36 (2012) 1107-1118. 10.1097/PAS.0b013e31825dd4b3
[13] C.B. Gilks, E. Oliva, R.A. Soslow, Poor interobserver reproducibility in the diagnosis of high-grade endometrial carcinoma. Am J Surg Pathol 37 (2013) 874-881. 10.1097/PAS.0b013e31827f576a
[14] G. Han, D. Sidhu, M.A. Duggan, J. Arseneau, M. Cesari, P.B. Clement, C.A. Ewanowich, S.E. Kalloger, M. Köbel, Reproducibility of histological cell type in high-grade endometrial carcinoma. Mod Pathol 26 (2013) 1594-1604. 10.1038/modpathol.2013.102
[15] R. Murali, B. Davidson, O. Fadare, J.A. Carlson, C.P. Crum, C.B. Gilks, J.A. Irving, A. Malpica, X. Matias-Guiu, W.G. McCluggage, K. Mittal, E. Oliva, V. Parkash, J.K.L. Rutgers, P.N. Staats, C.J.R. Stewart, C. Tornos, R.A. Soslow, High-grade Endometrial Carcinomas: Morphologic and Immunohistochemical Features, Diagnostic Challenges and Recommendations. Int J Gynecol Pathol 38 Suppl 1 (2019) S40-s63. 10.1097/pgp.0000000000000491
[16] S.K. Murray, R.H. Young, R.E. Scully, Unusual epithelial and stromal changes in myoinvasive endometrioid adenocarcinoma: a study of their frequency, associated diagnostic problems, and prognostic significance. Int J Gynecol Pathol 22 (2003) 324-333. 10.1097/01.pgp.0000092161.33490.a9
[17] J. Shia, D. Black, A.J. Hummer, J. Boyd, R.A. Soslow, Routinely assessed morphological features correlate with microsatellite instability status in endometrial cancer. Hum Pathol 39 (2008) 116-125. 10.1016/j.humpath.2007.05.022
[18] M. Saijo, K. Nakamura, N. Ida, A. Nasu, T. Yoshino, H. Masuyama, H. Yanai, Histologic Appearance and Immunohistochemistry of DNA Mismatch Repair Protein and p53 in Endometrial Carcinosarcoma: Impact on Prognosis and Insights Into Tumorigenesis. Am J Surg Pathol 43 (2019) 1493-1500. 10.1097/PAS.0000000000001353

[19] M.E. Sherman, P. Bitterman, N.B. Rosenshein, G. Delgado, R.J. Kurman, Uterine serous carcinoma. A morphologically diverse neoplasm with unifying clinicopathologic features. Am J Surg Pathol 16 (1992) 600-610. 10.1097/00000478-199206000-00008
[20] G. Han, R.A. Soslow, S. Wethington, D.A. Levine, F. Bogomolniy, P.B. Clement, M. Köbel, B. Gilks, D. DeLair, Endometrial Carcinomas With Clear Cells: A Study of a Heterogeneous Group of Tumors Including Interobserver Variability, Mutation Analysis, and Immunohistochemistry With HNF-1ß. Int J Gynecol Pathol 34 (2015) 323-333. 10.1097/pgp.0000000000000162
[21] M. Kobel, B. Tessier-Cloutier, J. Leo, L.N. Hoang, C.B. Gilks, R.A. Soslow, D. Delair, C.J.R. Stewart, C.H. Lee, Frequent Mismatch Repair Protein Deficiency in Mixed Endometrioid and Clear Cell Carcinoma of the Endometrium. Int J Gynecol Pathol 36 (2017) 555-561. 10.1097/pgp.0000000000000369
[22] S.F. Lax, E.S. Pizer, B.M. Ronnett, R.J. Kurman, Clear cell carcinoma of the endometrium is characterized by a distinctive profile of p53, Ki-67, estrogen, and progesterone receptor expression. Hum Pathol 29 (1998) 551-558. 10.1016/s0046-8177(98)80002-6
[23] L.N. Hoang, G. Han, M. McConechy, S. Lau, C. Chow, C.B. Gilks, D.G. Huntsman, M. Kobel, C.H. Lee, Immunohistochemical characterization of prototypical endometrial clear cell carcinoma--diagnostic utility of HNF-1beta and oestrogen receptor. Histopathology 64 (2014) 585-596. 10.1111/his.12286
[24] G.F. Zannoni, A. Santoro, G. Angelico, S. Spadola, D. Arciuolo, M. Valente, F. Inzani, A. Pettinato, S. Vatrano, F. Fanfani, G. Scambia, F. Fraggetta, Clear cell carcinoma of the endometrium: an immunohistochemical and molecular analysis of 45 cases. Hum Pathol 92 (2019) 10-17. 10.1016/j.humpath.2019.06.005
[25] M. Kobel, B. Meng, L.N. Hoang, N. Almadani, X. Li, R.A. Soslow, C.B. Gilks, C.H. Lee, Molecular Analysis of Mixed Endometrial Carcinomas Shows Clonality in Most Cases. Am J Surg Pathol 40 (2016) 166-180. 10.1097/pas.0000000000000536
[26] H.S. Bae, H. Kim, S. Young Kwon, K.R. Kim, J.Y. Song, I. Kim, Should endometrial clear cell carcinoma be classified as Type II endometrial carcinoma? Int J Gynecol Pathol 34 (2015) 74-84. 10.1097/pgp.0000000000000111
[27] N. Conlon, A. Da Cruz Paula, C.W. Ashley, S. Segura, L. De Brot, E.M. da Silva, R.A. Soslow, B. Weigelt, D.F. DeLair, Endometrial Carcinomas with a "Serous" Component in Young Women Are Enriched for DNA Mismatch Repair Deficiency, Lynch Syndrome, and POLE Exonuclease Domain Mutations. Am J Surg Pathol 44 (2020) 641-648. 10.1097/pas.0000000000001461
[28] D.E. Cohn, W.L. Frankel, K.E. Resnick, V.L. Zanagnolo, L.J. Copeland, H. Hampel,

N. Kelbick, C.D. Morrison, J.M. Fowler, Improved survival with an intact DNA mismatch repair system in endometrial cancer. Obstet Gynecol 108 (2006) 1208-1215. 10.1097/01.AOG.0000239097.42987.0c

[29] H.J. An, K.I. Kim, J.Y. Kim, J.Y. Shim, H. Kang, T.H. Kim, J.K. Kim, J.K. Jeong, S.Y. Lee, S.J. Kim, Microsatellite instability in endometrioid type endometrial adenocarcinoma is associated with poor prognostic indicators. Am J Surg Pathol 31 (2007) 846-853. 10.1097/01.pas.0000213423.30880.ac

[30] K.K. Shih, K. Garg, D.A. Levine, N.D. Kauff, N.R. Abu-Rustum, R.A. Soslow, R.R. Barakat, Clinicopathologic significance of DNA mismatch repair protein defects and endometrial cancer in women 40years of age and younger. Gynecol Oncol 123 (2011) 88-94. 10.1016/j.ygyno.2011.06.005

[31] D.S. McMeekin, D.L. Tritchler, D.E. Cohn, D.G. Mutch, H.A. Lankes, M.A. Geller, M.A. Powell, F.J. Backes, L.M. Landrum, R. Zaino, R.D. Broaddus, N. Ramirez, F. Gao, S. Ali, K.M. Darcy, M.L. Pearl, P.A. DiSilvestro, S.B. Lele, P.J. Goodfellow, Clinicopathologic Significance of Mismatch Repair Defects in Endometrial Cancer: An NRG Oncology/Gynecologic Oncology Group Study. J Clin Oncol 34 (2016) 3062-3068. 10.1200/jco.2016.67.8722

[32] K. Garg, M.M. Leitao, Jr., N.D. Kauff, J. Hansen, K. Kosarin, J. Shia, R.A. Soslow, Selection of endometrial carcinomas for DNA mismatch repair protein immunohistochemistry using patient age and tumor morphology enhances detection of mismatch repair abnormalities. Am J Surg Pathol 33 (2009) 925-933. 10.1097/PAS.0b013e318197a046

[33] B.E. Howitt, S.A. Shukla, L.M. Sholl, L.L. Ritterhouse, J.C. Watkins, S. Rodig, E. Stover, K.C. Strickland, A.D. D'Andrea, C.J. Wu, U.A. Matulonis, P.A. Konstantinopoulos, Association of Polymerase e-Mutated and Microsatellite-Instable Endometrial Cancers With Neoantigen Load, Number of Tumor-Infiltrating Lymphocytes, and Expression of PD-1 and PD-L1. JAMA Oncol 1 (2015) 1319-1323. 10.1001/jamaoncol.2015.2151

[34] D. Lim, P.P. Ip, A.N. Cheung, T. Kiyokawa, E. Oliva, Immunohistochemical Comparison of Ovarian and Uterine Endometrioid Carcinoma, Endometrioid Carcinoma With Clear Cell Change, and Clear Cell Carcinoma. Am J Surg Pathol 39 (2015) 1061-1069. 10.1097/pas.0000000000000436

[35] M. Heckl, E. Schmoeckel, L. Hertlein, M. Rottmann, U. Jeschke, D. Mayr, The ARID1A, p53 and ss-Catenin statuses are strong prognosticators in clear cell and endometrioid carcinoma of the ovary and the endometrium. PLoS One 13 (2018) e0192881. 10.1371/journal.pone.0192881

[36] G. Allo, M.Q. Bernardini, R.C. Wu, M. Shih Ie, S. Kalloger, A. Pollett, C.B. Gilks,

B.A. Clarke, ARID1A loss correlates with mismatch repair deficiency and intact p53 expression in high-grade endometrial carcinomas. Mod Pathol 27 (2014) 255-261. 10.1038/modpathol.2013.144
[37] T. Bosse, N.T. ter Haar, L.M. Seeber, P.J. v Diest, F.J. Hes, H.F. Vasen, R.A. Nout, C.L. Creutzberg, H. Morreau, V.T. Smit, Loss of ARID1A expression and its relationship with PI3K-Akt pathway alterations, TP53 and microsatellite instability in endometrial cancer. Mod Pathol 26 (2013) 1525-1535. 10.1038/modpathol.2013.96
[38] S.R. Kim, B.T. Cloutier, S. Leung, D. Cochrane, H. Britton, A. Pina, C. Storness-Bliss, D. Farnell, L. Huang, K. Shum, A. Lum, J. Senz, C.H. Lee, C.B. Gilks, L. Hoang, J.N. McAlpine, Molecular subtypes of clear cell carcinoma of the endometrium: Opportunities for prognostic and predictive stratification. Gynecol Oncol 158 (2020) 3-11. 10.1016/j.ygyno.2020.04.043
[39] H.J. An, S. Logani, C. Isacson, L.H. Ellenson, Molecular characterization of uterine clear cell carcinoma. Mod Pathol 17 (2004) 530-537. 10.1038/modpathol.3800057
[40] E.M. Miller, J. Tymon-Rosario, J. Sunkara, B.E. Harmon, R.G. Karabakhtsian, A.P. Novetsky, Comparative Use of Napsin A and Glypican 3 to Distinguish Endometrial Clear Cell from Serous and Endometrioid Carcinomas. Int J Gynecol Cancer 28 (2018) 1318-1324. 10.1097/igc.0000000000001303
[41] O. Fadare, M.M. Desouki, K. Gwin, K.Z. Hanley, E.A. Jarboe, S.X. Liang, C.M. Quick, W. Zheng, V. Parkash, J.L. Hecht, Frequent expression of napsin A in clear cell carcinoma of the endometrium: potential diagnostic utility. Am J Surg Pathol 38 (2014) 189-196. 10.1097/pas.0000000000000085
[42] M. Iwamoto, Y. Nakatani, K. Fugo, T. Kishimoto, T. Kiyokawa, Napsin A is frequently expressed in clear cell carcinoma of the ovary and endometrium. Hum Pathol 46 (2015) 957-962. 10.1016/j.humpath.2015.03.008
[43] J.A. Al-Maghrabi, N.S. Butt, N. Anfinan, K. Sait, H. Sait, A. Marzouki, M.N. Khabaz, Infrequent Immunohistochemical Expression of Napsin A in Endometrial Carcinomas. Appl Immunohistochem Mol Morphol 25 (2017) 632-638. 10.1097/pai.0000000000000350
[44] C.E. Matrai, E.C. Pirog, L.H. Ellenson, Despite Diagnostic Morphology, Many Mixed Endometrial Carcinomas Show Unexpected Immunohistochemical Staining Patterns. Int J Gynecol Pathol 37 (2018) 405-413. 10.1097/pgp.0000000000000443
[45] L. Vermij, V. Smit, R. Nout, T. Bosse, Incorporation of molecular characteristics into endometrial cancer management. Histopathology 76 (2020) 52-63. 10.1111/his.14015