Surgical and Experimental Pathology

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Primary intestinal type endometrial mucinous carcinoma: a case report and review of the literature

Sumru Cagaptay[1*], Tekincan Aktas[2], Tunc Timur[3], Zeynep Bayramoglu[1], Sefa Kurt[3] and Safiye Aktas[1,2]

Abstract

Primary gastric (gastrointestinal) mucinous carcinoma of the endometrium is a newly defined entity in the latest WHO classification. It is known to have an aggressive clinical course compared to conventional mucinous endometrial carcinomas. The case we present is a 65-year-old female patient. The patient underwent total abdominal hysterectomy, bilateral salpingooophorectomy, bilateral pelvic and para-aortic lymph node dissection, and omentectomy. Histologically, the tumor contains enterocyte-like cells with a brush border, as well as abundant eosinophilic cytoplasm with mild or moderate atypia and occasional goblet cells. In immunohistochemical evaluation, CK7, CK20, CDX2, Villin, and CEA was positive in the tumor. The patient was diagnosed as primary intestinal type mucinous endometrial carcinoma based on morphological and immunohistochemical findings. The patient's molecular examination revealed mutations in the TP53, RB, and FBXW7 genes. The patient has been followed up for 6 months without disease after receiving 6 cycles of chemotherapy. The clinical, histopathological, immunohistochemical, and molecular features of the case were described, and the literature was reviewed. The recognition and differential diagnosis of this rare tumor will contribute to appropriate patient management and accurate prognostication.

Keywords Endometrial cancer, Mucinous carcinoma, Intestinaltype mucinous carcinoma, Molecular features

Background

Endometrioid carcinoma is the most prevalent type of endometrial cancer. Since it shows a wide variety of morphologies and metaplastic changes, it might be challenging to diagnose. Mucinous differentiation is common in endometrioid carcinomas (Bartosch, Manuel Lopes and Oliva 2011; WHO Classification of Tumours Editorial Board 2020). They can rarely differentiate between intestinal and gastric types. It is rare to observe intestinal differentiation in endometrial cancer. The phenomenon was initially explained in 1984 by (Berger et al. 1984). Subsequently, additional cases of intestinal and gastric differentiation were documented in the literature (Abiko et al. 2010; Buell-Gutbrod et al. 2013; Hino et al. 2016; Karpathiou et al. 2022; McCarthy et al. 2017; Mogor et al. 2018; Rubio et al. 2016; Stolnicu et al. 2021; Trippel et al. 2017; Zheng et al. 1995). Due to its infrequency and the lack of agreement until recently, this tumor has been assigned various names by different writers and is frequently regarded as a Müllerian-type mucinous carcinoma. In 2020, (Wong et al. 2020) described four patients with endometrial cancer that had histologically unusual

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mucinous differentiation into a gastric (gastrointestinal) phenotype. Following this paper, the World Health Organization (WHO) classification of female genital tract cancers in 2020 included a new type of carcinoma: Primary gastric (gastrointestinal)–type endometrial mucinous carcinoma (WHO Classification of Tumours Editorial Board 2020). WHO classification defines this rare tumor as a carcinoma with mucinous gastric/gastrointestinal characteristics. Morphologically, it consists of glands formed by mucin-secreting epithelium, which may contain goblet cells with low grade nuclei. The exclusion of cervical origin and/or metastases from the gastrointestinal tract, the lack of an endometrioid component, and morphological characteristics are required (WHO Classification of Tumours Editorial Board 2020). The establishment of criteria has contributed to the recognition and description of new cases in recent years. However, the number of cases in the literature is still around 20 and the clinical, pathological and molecular features of the tumor have not been sufficiently elucidated. It is thought that recognition of this tumor type is important because it has been stated that it has an aggressive clinical course (Travaglino et al. 2020; Wong et al. 2020). With the case we present, we hope to add data to the literature that will increase the recognition of the tumor.

Case presentation

A 65-year-old female patient has a history of two spontaneous vaginal births. She has a history of appendectomy and thyroidectomy. She received radioactive iodine treatment with a diagnosis of thyroid papillary carcinoma. There is no feature in her family history. Endometrial curettage was performed on her application to another center with a complaint of postmenopausal bleeding. Endometrioid adenocarcinoma was reported as FIGO grade I. She was referred to our hospital for further examination. No abnormal findings were found in the physical examination performed in our hospital. In the radiological evaluation, a 5 mm irregular thickness increase was detected in the uterine fundus on transvaginal ultrasonography. Contrast-enhanced pelvic magnetic resonance imaging revealed an 8 mm segment of increased continuous wall thickness and endometrial irregularity within the myometrium of the uterine fundus. Consultation blocks of curettage material were re-evaluated preoperatively in our department. In microscopic evaluation, there is well-differentiated carcinoma morphology. The tumor consists of complex glandular structures composed of tall columnar cells with mild to moderate atypia (Fig. 1). In immunohistochemical examination, estrogen receptor (ER) and progesterone receptor (PR) are negative P53 displays a mutant type, while p16 exhibits patchy positive staining. The proliferation index for Ki-67 is high.

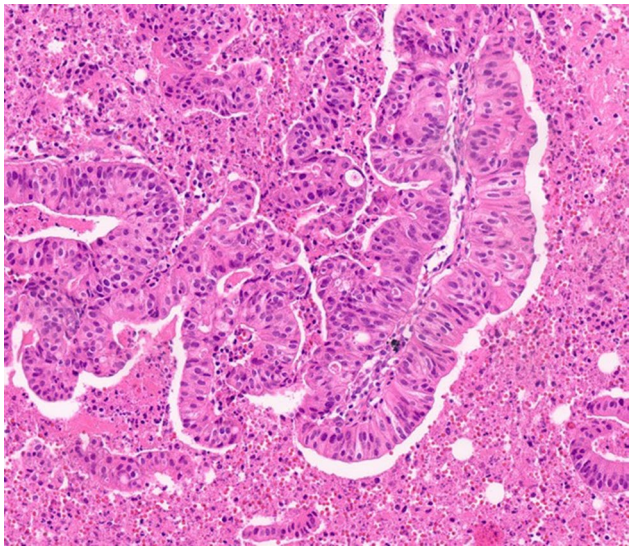


Fig. 1 Well-differentiated carcinoma morphology in curetage material. The tumor consists of complex glandular structures composed of tall columnar cells with mild to moderate atypia (H&E staining, x200)

Due to the unexpected immunophenotype of the tumor with low-grade morphology, human papilloma virus (HPV) Chromogenic In situ hybridization (ISH) was performed to investigate the possible cervical origin, and the result was negative. Material limitations prevented additional immunohistochemical examination for histological typing of the tumor at this stage. The patient underwent total abdominal hysterectomy and bilateral salpingooophorectomy, bilateral pelvic and para-aortic lymph node dissection, and omentectomy. In macroscopic evaluation, a tumor measuring 1.5x1 cm was found in the posterior wall of the uterus and showed an expansile growth pattern. The tumor invaded the upper half of the myometrium. No cervical extension was observed. Bilateral ovaries and fallopian tubes showed no tumor involvement. Histologically, the tumor contains enterocyte-like cells with a brush border, as well as abundant eosinophilic cytoplasm with mild or moderate atypia and occasional goblet cells (Fig. 2). Not accompanied by a typical endometrioid component. Adjacent to the tumor, we observe areas resembling high-grade intestinal type dysplasia and the non-tumoral endometrium was atrophic. The cervical gland and stroma showed no tumor involvement. The lymph nodes and omentum were histologically examined, and there was no tumor. In immunohistochemical evaluation (Fig. 3), ER, PR, PAX-8, WT-1, GATA-3, p40, and Napsin A were negative in the tumor. CK7, CK20, CDX2, Villin, and CEA was positive. There was focal positivity with AMACR. The tumor was negative with MUC-2 and MUC6 and stained weak positive with MUC5AC. P16 demonstrated block type positivity and p53 mutant type staining. The Ki-67 proliferation index was high. Additionally, MLH-1, MSH2, MSH6, and PMS2 mismatch

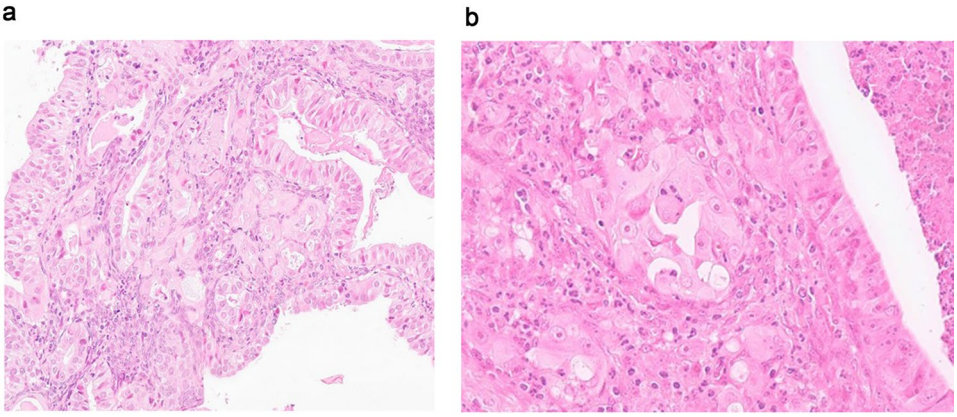


Fig. 2 **a**, **b**: enterocyte-like cells with a brush border, as well as abundant eosinophilic cytoplasm with mild or moderate atypia and occasional goblet cells (H&E staining, x200, H&E staining, x400, respectively)

repair (MMR) immunohistochemistry did not detect any loss of nuclear expression in the tumor.

The patient was also evaluated with endoscopy and colonoscopy for possible metastasis of a gastrointestinal system tumor, and no tumor focus was found. Morphological and immunohistochemical findings were evaluated together, and a diagnosis of primary intestinal type mucinous endometrial carcinoma was given. The tumor stage was determined as p T1bN0 (FIGO 2023 stage 1B).

Molecular analysis was also performed to understand the tumor pathogenesis and to provide a possible targeted therapy option. In molecular analysis, DNA and RNA were isolated from formalin-fixed paraffin-embedded tissue by microdissection of the lesion area for next-generation sequencing (NGS) and fusion detection. OncoReveal Multi-Cancer with CNV v4 Panel developed by Pillar Biosciences was used for NGS (for panel details; https://www.pillarbiosci.com/wp-content/uploads/2023/08/UM-0026-ONCOReveal-Multi-Cancer-RNA-Fusion-v2-Panel-6307-1.pdf). OncoReveal Multi-Cancer RNA Fusion v2 Panel developed by the same company was used for fusion detection (for panel details; https://www.pillarbiosci.com/wp-content/uploads/2023/08/UM-0028-ONCOReveal-Multi-Cancer-v4-with-CNV-Panel-6309-2.pdf). Following the isolation of genetic material, we processed the sample in accordance with Pillar Bioscience's instructions. The data threshold in the ClinVar database was used to interpret the detected variants to exclude known polymorphisms and identify pathogenicity.

There was no fusion detected in RNA fusion detection. NGS analysis performed on the sample detected 39 variants. 31 of these variants belong to intronic, 3'UTR or synonymous mutations. NM_000546.6:c0.817C > T (R273C missense) mutation in the TP53 gene was interpreted as pathogenic based on the data in ClinVar. NM_000455.5:c0.1062C > G (F354L missense) mutation in the STK11 gene and NM_000222.3:c0.1621A > C (M541L missense) mutation in the KIT gene were evaluated as benign. NM_004448.4:c0.1963A > G (I655V missense) mutation in the ERBB2 gene was interpreted as a variation due to its frequency in the population. NM_000222.3:c0.1454A > C (H485P missense) mutation in the KIT gene, NM_001349798.2:c0.1387A > G (T463A missense) and NM_033632.3:c0.1452 G > T(A484S missense) mutation in the FBXW7 gene, NM_000321.3:c0.2377C > T(P793S missense) mutation were detected in the RB1 gene. Since there was no data or sufficient data in ClinVar for these mutations, they were evaluated in the unknown significance category.

Considering the possibility of tumor recurrence in the postoperative treatment planning, it was decided to administer chemotherapy and radiotherapy to the patient. The patient received 6 cycles of carboplatin and pacitaxel combination treatment and has been followed up for 6 months without recurrence or metastasis.

Discussion

The diagnosis of gastrointestinal type endometrial carcinoma requires a comprehensive evaluation of all findings, as morphological and immunohistochemical features alone are not sufficient. When there is no expression of hormone receptors and no squamous morule in a low-grade endometrial carcinoma, and if the morphology supports it, the possibility of gastric (gastrointestinal) type endometrial carcinoma should be considered. According to the criteria defined by Wong et al. (Wong et al. 2020), the tumor is "(i) endometrial adenocarcinoma with no evidence of any other primary site and the presence of gastric-type morphology (voluminous, pale eosinophilic or clear cytoplasm with distinct cell borders) and/or goblet cells.

(ii) absence of typical endometrioid component (iii) absence of cervical glandular or stromal involvement (with complete sampling of the cervix) (iv) at least focal immunohistochemical expression of one or more gastrointestinal markers (eg, MUC6, CK20, and CDX2)

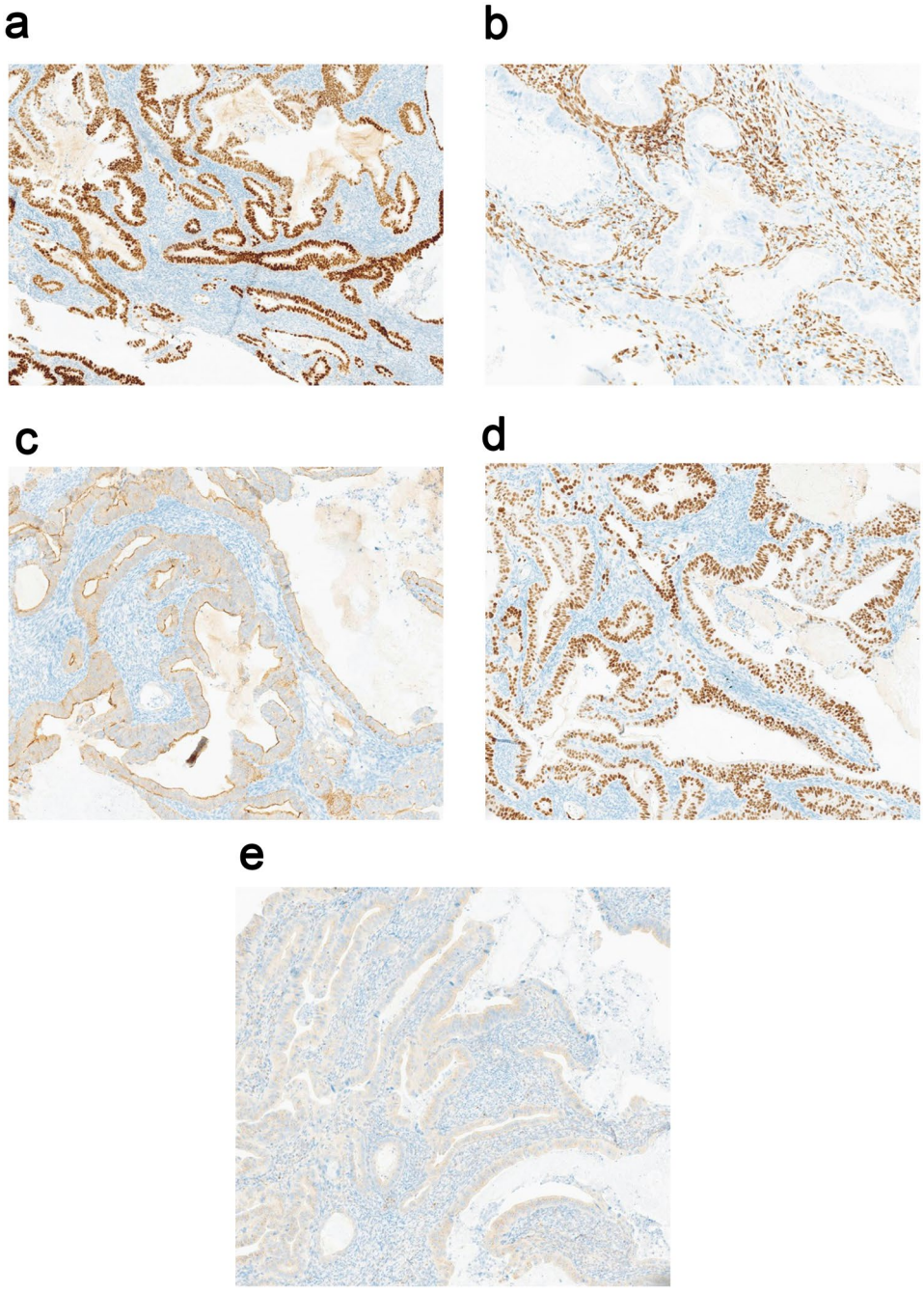


Fig. 3 **a**: mutant type staning at p53 immunohistochemistry, **b**: ER negativitiy in the tumor, **c**: positivity with villin immunohistochemistry, **d**: positivity with CDX2 immunohistochemistry, 3e: weak positivity with MUC5AC immunohistochemistry

(v) absent or minimal (< 5%) expression of ER" it can be diagnosed as gastrointestinal type endometrioid carcinoma.

Differential diagnoses include conventional (Müllerian) type endometrial mucinous carcinoma, gastric/intestinal type endocervical adenocarcinoma, endometrial metastases of a gastrointestinal malignancy, and benign lesions with gastrointestinal type mucinous metaplasia.

The latest WHO classification (WHO Classification of Tumours Editorial Board 2020) does not include the criterion of the 4th edition (CMHCYR, 2014) regarding the definition of conventional mucinous tumors, which states that "the tumor should be classified as mucinous carcinoma if it contains more than 50% mucinous component". This histological type is now included under the heading of other endometrial carcinomas as "Mucinous carcinoma, gastric (gastrointestinal) type". The terminology

of mucinous differentiation in endometrioid endometrial carcinomas has been retained, but no cut-off value has been specified (Parkash et al. 2023; WHO Classification of Tumours Editorial Board 2020). Therefore, the need for these distinctions arises not from the specification of conventional mucinous endometrial carcinomas as a distinct entity but rather from their superior prognosis compared to gastric (gastrointestinal) type mucinous carcinomas (CMHCYR, 2014; Wong et al. 2020; Wong, Talia and Mccluggage 2020). Morphologically, conventional -type mucinous carcinomas consist of endocervical type epithelium and do not have the cytomorphological and immunohistochemical features of gastric/gastrointestinal differentiation. They contain typical endometrioid components. It is generally diffusely positive with ER and PR (Shabani et al., 2007,Lax et al.,. 1998).

In curettage specimens, both HPV-related and HPV-unrelated endocervical adenocarcinomas can complicate differential diagnosis. Intestinal type endocervical adenocarcinomas are associated with HPV, and a significant proportion exhibit ISH positivity. In gastric type endocervical adenocarcinomas that are not associated with HPV, HPV ISH will yield negative results. While p16 block positivity may occasionally occur, this is less common compared to gastrointestinal type endometrial carcinoma. It is recommended to sample the entire cervix to rule out endometrial extension of primary endocervical adenocarcinoma (Dridi, Peoc'h and Karpathiou 2023; Stolnicu et al. 2018; Stolnicu, Hoang and Soslow 2019; Wong et al. 2020).

Since the morphological and immunohistochemical findings of endometrial metastasis from a tumor of gastrointestinal and pancreaticobiliary system origin may be very similar to gastrointestinal type endometrial carcinoma, evaluation of the patient together with clinical history, endoscopic and imaging findings may help determine the primary focus. Despite conflicting immunohistochemical findings, positivity with Müllerian markers may support gynecological origin (Choi et al. 2020; Dridi, Peoc'h and Karpathiou 2023; Ronquillo and Pinto 2022).

Benign lesions containing gastrointestinal type metaplasia can be distinguished from gastrointestinal type endometrial adenocarcinoma by the presence of ER expression, being generally associated with endometrial polyps, and not containing atypia (Wong et al. 2020).

We conducted a comprehensive review of the literature and compiled the cases presented to date (Additional file 1, summarizes the findings in cases of endometrial adenocarcinoma with gastric (gastrointestinal) type differentiation described in the literature).

Morphologically, the cases presented in the literature review to date consist of cases that generally show a glandular pattern, and less frequently a papillary, cribriform and solid pattern. Two cases describe tumor cells that form small aggregates or small fused glands within mucinous pools (Rubio et al. 2016; Trippel et al. 2017). In our case, the tumor consists predominantly of glandular and cribriform patterns.

In the cases presented, gastric (Abiko et al. 2010; McCarthy et al. 2017; Travaglino et al. 2020; Wong et al. 2020; Wong, Talia and Mccluggage 2020) and intestinal (Ardighieri et al. 2020; Berger et al. 1984; Buell-Gutbrod et al. 2013; Nakano et al. 2023; Trippel et al. 2017; Zheng et al. 1995) type differentiation, as well as gastrointestinal type differentiation (Karpathiou et al. 2022; Rubio et al. 2016; Stolnicu et al. 2021), were observed. According to Wong et al. (Wong et al. 2020), the term ''gastrointestinal type differentiation" refers to cases where the differentiation is not pure and of a single type but includes both gastric and intestinal features. When morphologically gastric/intestinal/gastrointestinal differentiation is suspected, intestinal (CDX2, CK20) and gastric differentiation markers (MUC6, HIK1083) may be useful (Dridi, Peoc'h and Karpathiou 2023; Wong et al. 2020). However, since these markers can also be positive in conventional endometrioid adenocarcinomas (Alameda et al. 2007; Hodgson, Parra-Herran and Mirkovic 2019), it is recommended to evaluate cytomorphological features together with immunohistochemical features (Wong et al. 2020). In our case, CDX2 and CK20 positivity and MUC6 negativity were interpreted as intestinal differentiation considering the cytomorphological features.

The level of atypia is defined as mild and moderate in most cases and severe in some cases (Karpathiou et al. 2022; Travaglino et al. 2020; Wong et al. 2020). In most cases, the tumor appears to contain goblet cells and enterocyte-like tall columnar cells. In some cases, the tumor has been reported to contain varying amounts of neuroendocrine cells (Berger et al. 1984; Zheng et al. 1995), Paneth cells (Berger et al. 1984), and signet ring cells (Berger et al. 1984; Travaglino et al. 2020). Tumor may exhibit morphological heterogeneity. Contrary to the established criteria, cases with an accompanying endometrioid (Ardighieri et al. 2020; Berger et al. 1984; Buell-Gutbrod et al. 2013; McCarthy et al. 2017) or serous (Travaglino et al. 2020) component have been reported. In the case we present, similar to most cases in the literature, the tumor consists of enterocyte-like cells with a brush border, abundant eosinophilic cytoplasm with mild to moderate atypia, and occasional goblet cells, and does not contain a typical endometrioid component.

Some lesions that may have originated from or been associated with the tumor have been described in the literature. The lesions identified are atypical endometrial hyperplasia (Buell-Gutbrod et al. 2013; Mogor et al. 2018), mucinous adenocarcinoma in situ and mucinous metaplasia (Abiko et al. 2010), intestinal metaplasia in endometrial polyp (Rubio et al. 2016), gastric metaplasia

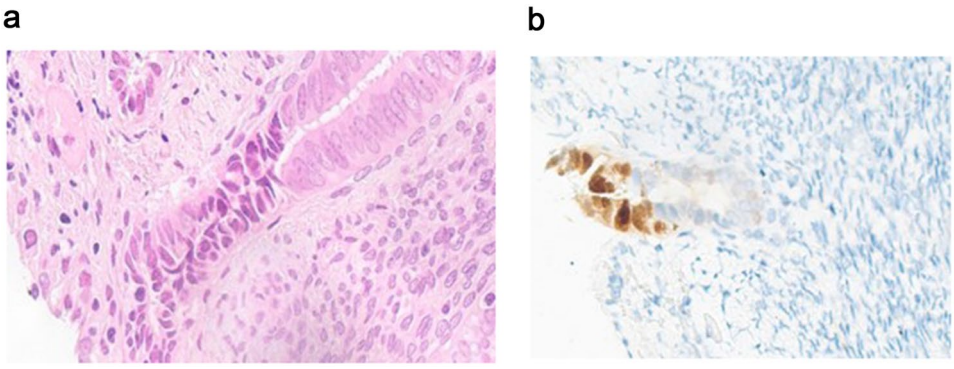


Fig. 4 Transition between areas of high-grade intestinal dysplasia and non-dysplastic epithelium **a**: H&E staining, x400, **b**: mutant-type staining of dysplastic areas in p53 immunohistochemistry

and low-grade dysplasia accompanying lobular glandular hyperplasia (Travaglino et al. 2020), mucinous gastric type metaplasia in adenomyomatous polyp and adenocarcinoma in situ on the basis of müllerian malformation (Wong et al. 2020). Metaplastic lesions that are associated with the tumor have been observed in certain cases. Additionally, there is a study in the literature that indicates that endometrial adenocarcinomas are associated with multifocal and synchronous gastric metaplastic lesions in the female genital tract (Mikami et al. 2009).

In the case we present, the non-neoplastic endometrium is atrophic, does not contain metaplastic benign lesions, and areas resembling high-grade intestinal dysplasia are observed adjacent to the tumor. It seems appropriate to use the terminology of "atypical gastrointestinal type mucinous proliferation" (Fig. 4) for this lesion, as it meets the criteria defined by Wong et al. (Wong et al. 2020). However, due to the wide range of potential tumor-related or precursor lesions identified and the small number of cases, it is unclear whether these lesions are true precursor lesions, and there is no consensus on this issue in the literature. It is believed that additional data are needed to understand this issue.

The immunohistochemistry panels utilized in the cases presented in the literature exhibited variations. Upon reviewing the findings based on the common markers utilized, positivity was observed in most cases for CK7, CK20, and CDX2. In two cases, CDX2 was negative (Wong et al. 2020). Villin positivity was observed in all cases where villin was administered (Rubio et al. 2016; Wong et al. 2020). PAX8 was positive in some cases and negative in others (Karpathiou et al. 2022; Mogor et al. 2018). In the majority of cases, ER and PR were reported as negative, while focally positive in a few cases and weakly positive in one case (Zheng et al. 1995). Only two of the cases where CEA was used yielded negative results (McCarthy et al. 2017). MUC6 was the most used mucin immunohistochemical marker, with positive results. In one case, all three mucin immunohistochemistry tests were performed, and the result was found to be positive for all three markers (Rubio et al. 2016). In p16 immunohistochemistry, there have been cases of both block (Karpathiou et al. 2022; Stolnicu et al. 2021; Wong et al. 2020; Wong, Talia and Mccluggage 2020) and patchy type positivity (Ardighieri et al. 2020; McCarthy et al. 2017; Rubio et al. 2016). There have been cases where p53 immunohistochemistry has revealed both mutant (Karpathiou et al. 2022; Wong et al. 2020) and wild type (Stolnicu et al. 2021; Travaglino et al. 2020; Wong et al. 2020) staining. HER2 immunohistochemistry was performed on only one of the cases, and the result was negative (Wong et al. 2020). Similar to the cases in the literature, CDX2, CK20, CK7, villin were positive and ER, PR were negative in our case. MUC2 and MUC6 were negative. Like only one case presented in the literature, MUC5AC was positive (Rubio et al. 2016). MLH1, PMS2, MSH6, and MSH2 nuclear expression were preserved (Karpathiou et al. 2022; McCarthy et al. 2017; Stolnicu et al. 2021) in three of the six cases in which MMR immunohistochemistry was utilized. In two cases, nuclear expression of MLH1 and PMS2 was lost, and molecular analysis revealed MLH1 hypermethylation (McCarthy et al. 2017; Trippel et al. 2017). In a single case, MSH6 and MSH2 were reported to have lost nuclear expression. In this case, molecular analysis confirmed the MMR defect/MSI-H status (Ardighieri et al. 2020). Loss of nuclear expression of MMR markers was not observed in our case.

The molecular pathogenesis of gastric (gastrointestinal) type endometrial carcinoma is not well understood. Molecular features have been described in only a small fraction of cases presented in the literature. In the case series by Wong et al., a SMARCB1 missense mutation and a RB1 nonsense mutation were described in one case. In the case with RB1 mutation, it was stated that the RB gene from the HPV-unrelated pathway may be effective in pathogenesis due to the presence of block type p16 positivity. In two cases with mutant type staining with p53, it was reported that the TP53 gene may be

responsible for pathogenesis (Wong et al. 2020). In three cases, pathogenesis was associated with MMR defect (Ardighieri et al. 2020; McCarthy et al. 2017; Trippel et al. 2017). It has been stated that mutations in the STK11 tumor suppressor gene may be effective in pathogenesis in cases accompanied by synchronous mucinous metaplastic lesions of the female genital tract, with or without Peutz-Jeghers syndrome (Mikami et al. 2009).

Our molecular analysis results show that the TP53 and RB genes play an important role in pathogenesis. Although there is insufficient data for mutations in the FBXW7 gene in ClinVar, the literature reports that the T463A mutation is moderately effective in serous endometrial carcinomas and the A484S mutation is highly effective in endometrial carcinomas (Le et al. 2012). The role of the FBXW7 gene in the pathogenesis of gastric (gastrointestinal) type endometrial carcinomas is not yet known, but it is thought that both mutations may be effective in pathogenesis since they affect the WD repeat domain. The combination of TP53, RB1, and FBXW7 mutations detected in our case has not been previously reported in the literature. However, this combination of mutations seems to clarify the aggressive nature of the tumor.

Conclusion

Recognizing and making a differential diagnosis of this tumor, known for its aggressive clinical course despite its low-grade morphological features, is crucial for appropriate clinical management and treatment preference (Travaglino et al. 2020; Wong et al. 2020; Wong, Talia and Mccluggage 2020). Although the histomorphological and immunohistochemical features of this tumor are relatively well understood, there is insufficient data in the literature regarding its molecular characteristics and biological behavior. In the case we present, we identified a combination of TP53, RB1, and FBXW7 mutations, which are rare but which we believe are associated with the aggressive course of the tumor. The incorporation of additional cases in the literature will enhance our comprehension of the tumor's characteristics.

Abbreviations

WHO	World Health Organization
ER	Estrogen receptor
PR	Progesterone receptor
HPV	Human papilloma virus
ISH	In situ hybridization
MMR	Mismatch repair
NGS	Next-generation sequencing

Supplementary Information

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Supplementary Material 1

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Not applicable.

Author contributions

Authors SC and ZB were responsible for patient data collection and preparation of the manuscript. Authors TA and SA performed the molecular analyses and analyzed the results. Authors TT and SK provided their clinical expertise. Authors ZB and SA critically revised the manuscript for important intellectual content. All authors approved the final manuscript and agreed to be responsible for all aspects of the work and to ensure that any questions about the work's accuracy or integrity are properly investigated and resolved.

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Data availability

The data generated and analyzed during the current study are not publicly available due to their personal data content but are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Our institution does not require ethical approval for reporting individual cases or case series. The research was conducted in accordance with the International Committee of Medical Journal Editors guidelines and the Helsinki Declaration as revised in 2013. All nonessential identifying details have been omitted.

Consent for publication

Informed consent was obtained from the patient. The participant has

Competing interests

The authors declare no competing interests.

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