Pathology & Oncology Research | frontiers

TYPE Original Research
PUBLISHED 19 November 2026
DOI 10.3389/pore.2026.1612616

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OPEN ACCESS

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RECEIVED 04 September 2026
REVISED 03 October 2026
ACCEPTED 27 October 2026
PUBLISHED 19 November 2026

CITATION
Betenbuk J, Vereczkey I, Szalai L, Simon A, Tóth E, Kuthi L and Melegh Z (2026) Molecular characterisation of mixed and dedifferentiated endometrial carcinomas reveals shared pathogenesis and clonal origin.
Pathol. Oncol. Res. 32:1612616.
doi: 10.3389/pore.2026.1612616

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Molecular characterisation of mixed and dedifferentiated endometrial carcinomas reveals shared pathogenesis and clonal origin

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Objectives: Mixed carcinoma of the endometrium (MCE) is an epithelial malignancy composed of two or more histological types. The recent WHO classification of MCE differs from its ovarian counterpart: dedifferentiated carcinomas are excluded from MCE, whereas ovarian dedifferentiated carcinomas are considered a special type of mixed carcinoma. However, the genetic basis of MCE remains poorly understood, as studies with comprehensive genomic analyses are scarce. This study aimed to identify genetic alterations involved in the development of MCE and endometrial dedifferentiated carcinomas (EDC) using next-generation sequencing (NGS) data and to determine whether MCE and EDC show distinct pathogenetic features supporting their classification as separate entities.

Methods: We retrospectively reviewed 1,578 endometrial carcinomas treated at or referred to our institute between January 2017 and December 2025. Mixed carcinomas were identified by morphological examination and immunohistochemistry. NGS using the Oncomine Comprehensive Assay V3 was performed separately on each component of six MCEs. Although EDCs are not classified as mixed carcinomas according to WHO criteria, nine such cases were also analysed for comparison.

Results: Excluding carcinosarcomas, MCEs accounted for 0.63% (10/1,578) of endometrial carcinomas, while EDCs represented 0.57% (9/1,578). With one exception, all MCEs and EDCs shared at least one identical pathogenic mutation between their components, supporting a clonal relationship. Among MCEs, 60% (6/10) showed mismatch repair deficiency (dMMR) in at least one component, and one case harboured a *POLE* mutation. Similarly, 89% (8/9) of EDCs were dMMR in at least one component, while one case was *POLE*-mutated. Both tumour groups frequently harboured alterations in the SWI/SNF and PI3K/AKT pathways, particularly involving *ARID1A*, *PIK3CA*, and *PTEN*. *ARID1A* mutations were especially frequent in EDCs (7/9, 78%).

Conclusion: The components of almost all MCE and EDC were clonally related, suggesting development through transdifferentiation or divergent differentiation rather than independent collision. Despite their classification as separate entities in the current WHO system, both tumour types appear to arise predominantly in

the setting of dMMR and genomic hypermutability. These findings provide further insight into the molecular pathogenesis of mixed carcinomas of the female genital tract and may contribute to future refinements in their classification.

KEYWORDS

dedifferentiated endometrial carcinoma, divergent differentiation, mismatch repair deficiency, mixed endometrial carcinoma, next generation sequencing

Introduction

Mixed carcinomas of the female genital tract are defined as carcinomas comprising two or more distinct histological subtypes. Mixed carcinomas of the endometrium (MCE) are specifically recognised in the World Health Organization (WHO) Classification of Female Genital Tumours 5th edition, where they are defined as carcinomas containing at least one component of either serous or clear cell carcinoma. The diagnosis requires the identification of at least two spatially distinct histological types of endometrial carcinoma by histology and immunohistochemistry [1]. MCEs are rare, reported to represent less than 3% of endometrial malignancies. The most common mixed histological types are endometrioid/serous, serous/clear cell, and endometrioid/clear cell carcinoma [2]. It is recommended that the proportion of each component be reported, as even a small proportion of a high-grade component can result in unfavourable prognosis and influences therapeutical decisions [1–4].

Although the diagnosis is primarily based on morphological features, additional immunohistochemical studies may support the identification of distinct components. This diagnostic category should not, however, be applied to morphological variations of endometrioid, clear cell carcinoma, serous carcinoma, to carcinomas with ambiguous morphology, endometrial dedifferentiated carcinomas (EDCs), and carcinosarcomas [1, 5]. Although the WHO classification excludes EDCs from the category of MCE, the most common type of endometrial carcinoma with multiple components is EDC (well-differentiated carcinoma with an undifferentiated carcinoma component). This exclusion of EDCs from the category of MCE differs from that of ovarian carcinomas, where EDCs are included within the mixed carcinoma category in the current WHO classification [1].

Based on genetic, epigenetic, and proteomic analyses, The Cancer Genome Atlas (TCGA) have proposed a molecular classification scheme for endometrial carcinomas [6]. According to this classification, endometrial carcinomas can be divided into four molecular groups: *POLE*-mutated (ultramutated), mismatch repair deficient (dMMR, hypermutated), *TP53*-mutated, and no specific molecular profile (NSMP) groups. However, there is limited data on the development, molecular background, and classification of mixed carcinomas.

Conceptually, mixed carcinomas may arise through three different mechanisms. These tumours can theoretically develop through divergent differentiation from a common progenitor, via transdifferentiation, or as collision tumours. It has been suggested that most MCEs arise through divergent differentiation and transdifferentiation, while true collision tumours are rare [1, 5, 7, 8]. However, to the best of our knowledge, only a limited number of small gene panel studies and case reports investigating the molecular characteristics of MCEs have been published.

The aim of this study was therefore two-fold: first, to identify common factors involved in the development of MCE, and second, to investigate whether pathogenetic differences exist between mixed and EDCs that would substantiate their classification as separate diagnostic categories within the endometrium. We therefore reviewed all MCE and EDC cases reported at our institute between January 2017 and December 2025 and separately analysed the tumour components and morphologically distinct areas using a next-generation sequencing (NGS) panel.

Materials and methods

Case selection

Between January 2017 and December 2025, 1,578 endometrial carcinomas were treated or referred for consultation to the National Institute of Oncology, Budapest, Hungary. Cases were classified as MCEs when at least two spatially distinct histological types were identified on morphology and immunohistochemistry, according to the current WHO criteria. We also identified EDCs, defined as tumours comprising a differentiated component (typically of FIGO grade 1 or 2 endometrioid carcinoma) and an undifferentiated component. Mixed tumours containing a pure mesenchymal component (carcinosarcoma) were excluded.

The different components were manually dissected, and DNA was extracted separately from each component of six MCEs and nine EDCs. Molecular analysis of each component was performed on the resection specimen, if available. In a few cases, certain components were only identifiable in the preoperative sample. In these cases, molecular analysis of these components was performed on the preoperative specimen. NGS was performed on each component.

Immunohistochemistry (IHC)

For each case, a basic panel including antibodies for oestrogen receptor (ER), progesterone receptor (PR), mismatch repair proteins (PMS2, MLH1, MSH6, and MSH2), and p53 was applied. In addition, for identification of a clear cell carcinoma component, NapsinA, AMACR (p504S), and HNF1-beta immunohistochemistry were performed. In those cases where a serous carcinoma component was present, p16 was ordered, and in EDCs, PAX8 and CK8-18 IHCs were added to the panel. Antibody clones and dilutions are listed in Supplementary Table S1. Each IHC reaction was performed according to the manufacturers' instructions and was evaluated in accordance with the College of American Pathologists' "Template for Reporting Results of Biomarker

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TABLE 1 Histological, immunophenotypical characteristics, and TCGA group classification of each component in mixed and dedifferentiated endometrial carcinomas.

Case number	Histology	ER (%)	PR (%)	p53	MMR proteins	NapsinA	AMACR	HNFb	p16	NGS	TCGA MPGs	Final TCGA MPG
Mixed endometrial carcinomas												
Case M1	Clear cell carcinoma	0	0	Wild type	Retained	Focal	Focal	NP	NP	Yes	*POLE* mutated	*POLE* mutated
	Low grade endometrioid carcinoma	50	10	Wild type	Retained	NP	NP	NP	NP		*POLE* mutated	*POLE* mutated
Case M2	Clear cell carcinoma	0	0	Wild type	Retained	Positive	Positive	Positive	Positive	Yes	NSMP	NSMP
	Low grade endometrioid carcinoma	90	90	Wild type	PMS2 and MLH1 loss	Negative	Negative	Negative	Negative		MMR deficient	MMR deficient
Case M3	Clear cell carcinoma	0	0	Wild type	MSH6 loss	Positive	NP	Positive	NP	Yes	MMR deficient	MMR deficient
	Low grade endometrioid carcinoma	100	70	Wild type	PMS2 and MLH1 loss	NP	NP	NP	NP		MMR deficient	MMR deficient
Case M4	Clear cell carcinoma	70	0	Wild type	MSH6, MSH2 loss	Positive	NP	Positive	NP	Yes	MMR deficient	MMR deficient
	Low grade endometrioid carcinoma	10	15	Wild tpye	MSH6, MSH2 loss	Negative	NP	NP	Focal		MMR deficient	MMR deficient
Case M5	Serous carcinoma	0	0	Aberrant (overexpression)	PMS2 and MLH1 loss	NP	NP	NP	Diffuse	Yes	MMR deficient/ p53-aberrant	MMR deficient
	High grade endometrioid carcinoma	100	90	Wild type	PMS2 and MLH1 loss	NP	NP	NP	Focal		MMR deficient	MMR deficient
Case M6	Clear cell carcinoma	90	50	Wild type	PMS2 loss	Positive	NP	NP	NP	Yes	MMR deficient	MMR deficient
	High grade endometrioid carcinoma	90	50	Wild type	PMS2 and MLH1 loss	NP	NP	NP	NP		MMR deficient	MMR deficient
Case M7	Serous carcinoma	100	100	Aberrant (overexpression)	Retained	NP	NP	NP	NP	No	NA	NA
	Low grade endometrioid carcinoma	100	100	Wild type	Retained	NP	NP	NP	NP		NA	NA
Case M8	Serous carcinoma	70	60	Aberrant (overexpression)	Retained	NP	NP	NP	NP	No	NA	NA
	Low grade endometrioid carcinoma	90	80	Wild type	Retained	NP	NP	NP	NP		NA	NA

(*Continued*)

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TABLE 1 Continued

Case number	Histology	ER (%)	PR (%)	p53	MMR proteins	NapsinA	AMACR	HNFb	p16	NGS	TCGA MPGs	Final TCGA MPG
Case M9	Serous carcinoma	80	0	Aberrant (overexpression)	Retained	Negative	NP	NP	NP	No	NA	NA
	Clear cell carcinoma	0	0	Wild type	Retained	Positive	NP	NP	NP		NA	NA
Case M 10	Clear cell carcinoma	0	0	Wild type	PMS2 and MLH1 loss	Negative	Negative	NP	NP	No	NA	NA
	High grade endometrioid carcinoma	13	2	Wild type	PMS2 and MLH1 loss	NP	NP	NP	NP		NA	NA
Dedifferentiated carcinomas												
	Histology	ER (%)	PR (%)	p53	MMR	CK8-18	PAX8	NP	NP	NGS	TCGA MPGs	Final TCGA MPG
Case D1	Undifferentiated carcinoma	0	0	Wild type	PMS2 and MLH1 loss	Focal	Negative	NP	NP	Yes	MMR deficient	MMR deficient
	Low grade endometrioid carcinoma	95	50	Wild type	PMS2 and MLH1 loss	Positive	Positive	NP	NP		MMR deficient	MMR deficient
Case D2	Undifferentiated carcinoma	0	0	Wild type	PMS2 and MLH1 loss	Focal	Negative	NP	NP	Yes	MMR deficient	MMR deficient
	Low grade endometrioid carcinoma	100	100	Wild type	Retained	NP	Positive	NP	NP		NSMP	NSMP
Case D3	Undifferentiated carcinoma	0	0	Wild type	PMS2 and MLH1 loss	Focal	Negative	NP	NP	Yes	MMR deficient	MMR deficient
	Low grade endometrioid carcinoma	100	90	Wild type	PMS2 and MLH1 loss	NP	NP	NP	NP		MMR deficient	MMR deficient
Case D4	Undifferentiated carcinoma	0	0	Wild type	MSH6, MSH2, PMS2, and MLH1 loss	Focal	Negative	NP	NP	Yes	MMR deficient	MMR deficient
	Low grade endometrioid carcinoma	100	70	Wild type	PMS2 and MLH1 loss	Positive	Positive	NP	NP		MMR deficient	MMR deficient
Case D5	Undifferentiated carcinoma	0	0	Subclonal aberrant (overexpression)	PMS2 and MLH1 loss	NP	NP	NP	NP	Yes	MMR deficient/ p53-aberrant	MMR deficient
	Low grade endometrioid carcinoma	90	90	Wild type	PMS2 and MLH1 loss	NP	NP	NP	NP		MMR deficient	MMR deficient
Case D6	Undifferentiated carcinoma	0	0	Wild type	PMS2 and MLH1 loss	Focal	Negative	NP	NP	Yes	MMR deficient	MMR deficient
	Low grade endometrioid carcinoma	100	90	Wild type	PMS2 and MLH1 loss	NP	NP	NP	NP		MMR deficient	MMR deficient

(*Continued*)

TABLE 1 Continued

Case number	Histology	ER (%)	PR (%)	p53	MMR proteins	NapsinA	AMACR	HNFb	p16	NGS	TCGA MPGs	Final TCGA MPG
Case D7	Undifferentiated carcinoma	0	0	Wild type	PMS2, MLH1, and MSH6 loss	Focal	Negative	NP	NP	Yes	MMR deficient	MMR deficient
	Clear cell carcinoma	0	0	Wild type	MSH6 loss	Positive	Positive	NP	NP		MMR deficient	MMR deficient
Case D8	Undifferentiated carcinoma	0	0	Wild type	Retained	Positive	Negative	NP	NP	Yes	*POLE* mutated	*POLE* mutated
	Low grade endometrioid carcinoma	100	100	Wild type	Retained	Positive	Positive	NP	NP		*POLE* mutated	*POLE* mutated
Case D9	Undifferentiated carcinoma	0	0	Wild type	PMS2 and MLH1 loss	Positive	Negative	NP	NP	Yes	MMR deficient	MMR deficient
	High grade endometrioid carcinoma	90	90	Wild type	PMS2 and MLH1 loss	Positive	Positive	NP	NP		MMR deficient	MMR deficient

NP: not performed; NA: not applicable; MPG: molecular profile group. Where there are multiple TCGA, molecular profile groups (double classifier) present within the tumo rs, these are listed in the TCGS MPGs coloumn, while the final classification, based on the TCGA guidelines and PROMISE algorithm is listed in the Final TCGA MPG column [7].

Testing of Specimens from Patients with Carcinoma of Gynecologic Origin" [9].

Nucleic acid extraction

Prior to macrodissection, the area of interest was identified and marked by a pathologist on haematoxylin and eosin-stained slides. Macrodissection of the components was performed using the marked areas as a guide, processing one to three 5 μm sections from the formalin-fixed, paraffin-embedded tissue blocks. DNA was extracted using the ReliaPrep DNA Clean-Up and Concentration System Kit (Promega, Madison, WI, United States) according to the manufacturer's protocol. As this system contains only purifying reagents, the deparaffinization and lysis steps were performed using the corresponding reagents according to the Maxwell RSC DNA formalin-fixed, paraffin-embedded protocol (Promega). The concentration of the DNA samples was measured using a Qubit 4 fluorometer with a Qubit™ 1X dsDNA HS (High Sensitivity) Assay Kit (Thermo Fisher Scientific, Waltham, MA, United States) and stored at 4 °C until further use.

Mutation detection methods

Libraries were prepared using the Ion Chef™ System with Ion 540™ Chips (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions with a DNA input of approximately 8.5 ng. Sequencing was performed using an Ion S5™ Plus Sequencer (Thermo Fisher Scientific, Waltham, MA, USA). Ion Reporter™ Software (v. 5.18) (Thermo Fisher Scientific, Waltham, MA, USA) was used for data analysis. Oncomine Comprehensive Assay v3 DNA GX was used as the analysis workflow for all the samples. Only those detected mutations classified as pathogenic or likely pathogenic were included for the purpose of this study.

Results

Based on morphology and IHC, the overall frequency of MCEs was 0.63% (10 out of 1578 cases), while 0.57% (9 out of 1578 cases) for EDCs. Six patients with MCE (Cases M1-M6) and all 9 patients with EDCs were further analysed by NGS. In the remaining four patients with MCE, further NGS analysis could not be performed, as no material was available for nucleic acid extraction (Tables 1, 2; Supplementary Table S2). Among the MCEs, clear cell carcinoma admixed with low-grade endometrioid carcinoma was the most common histological type (4 cases). Additional combinations were serous carcinoma admixed with low-grade endometrioid carcinoma, and clear cell carcinoma with high-grade endometrioid carcinoma (2 cases each) (Figure 1), serous carcinoma admixed with clear cell carcinoma, and serous carcinoma with high-grade endometrioid carcinoma (1 case each) (Figure 2).

Regarding the genotype of the tumour components, all MCEs and EDCs, apart from one case (Case M2), had at least one identical pathogenic mutation shared in both components. In Case M2, no pathogenic mutation was detected in the clear cell carcinoma component. This might be due to low tumour cell content or a result of an analytical error. In two MCE and one EDC patients, all components had the same mutational profile with the NGS panel used (Table 2).

Out of the 10 MCEs, 5 showed dMMR phenotype in both components, while there was a single case with dMMR in only one component on IHC. These dMMR cases were predominantly tumours with clear cell carcinoma and endometrioid carcinoma components (5 cases), with only one case (Case M5) comprising serous and endometrioid carcinoma components. Pathogenic *POLE* mutation was observed in one case, in both clear cell and low-grade endometrioid carcinoma components of the tumour (Case M1). Aberrant p53 expression (diffuse nuclear overexpression) was detected in the serous component of four cases (Cases M5, M7, M8, and M9) (4/10). NGS analyis was performed only on Case M5,

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TABLE 2 Pathogenic mutations detected in each component in mixed and dedifferentiated endometrial carcinoma.

Case number	Histology	1st component	2nd component
Mixed endometrial carcinomas			
Case M1	CCC/LGEC	*POLE, ARID1A, PTEN, PIK3A, XPO1, IDH1*	
Case M2	CCC/LGEC		*KRAS, PIK3CA, PTEN, NFE2L2*
Case M3	CCC/LGEC	*PIK3CA*	
		ARID1A, FGXW7	
		MSH6, KRAS, ATM, SMAD4	*PTEN, FGFR2, CCND1, ERBB3*
Case M4	CCC/LGEC	*MSH6, PIK3CA, PPP2R1A, ARID1A*	
Case M5	SC/HGEC	*PALB2, PTEN, MAPK*	
		TP53, RB1	*ATM*
Case M6	CCC/HGEC	*PTEN*	
		PIK3CA	
		NRAS, ATM	
Dedifferentiated carcinomas			
Case D1	UC/LGEC	*KRAS, ARID1A, PTEN, CCND1*	
		PIK3R1	
			RAD50, MED12
Case D2	UC/LGEC	*AKT1, PIK3CA, CTNNB1, MAP2K2*	
		PIK3CA, CCND1	
Case D3	UC/LGEC	*PIK3CA, KRAS, ARID1A*	
Case D4	UC/LGEC	*FGR2, ARID1A, PTEN*	
		CTNNB1	
Case D5	UC/LGEC	*FGFR2, PTEN, PIK3CA*	
		TP53	*PMS2*
Case D6	UC/LGEC	*PTEN, ARID1A*	
		PIK3CA	
		SMARCA4, PIK3R1	
Case D7	UC/CCC	*MSH6*	
		ATM, PTEN, ARID1A, NF1	
		ERBB2, APC, KMT2D, PIK3R1, SPOP	*PIK3CA, IDH1, PPP2R1A, TP53, FANCI, SMAD4*
Case D8	UC/LGEC	*POLE, BRCA2, RAD50, ARID1A, NF1, FBXW7*	
		SETD2, BRCA2, RAD50, ARID1A	
		BRCA1, NF2, RB1, PIK3R1, CREBBP, ATRX, KIT	*PIK3CA, ATM, SLX4*

(*Continued*)

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TABLE 2 Continued

Case number	Histology	1st component	2nd component
Case D9	UC/HGEC	*KRAS, MAX, PTEN*	
		PIK3CA	
		MYC, CTNNB1, BRAF, ATM, RNF43, ARID1A, CCND1, CDKN2A, CCND2	

Abbreviations: CCC, clear cell carcinoma; LGEC, low grade endometrioid carcinoma; SC, serous carcinoma; HGEC, high grade serous carcinoma; UC, undifferentiated carcinoma. Blue: the same pathogenic mutations are present in both components. Orange: both components harboured pathogenic mutations in the same genes, although the specific mutations differed. Grey: mutations present only in one component.

where a pathogenic *TP53* mutation was confirmed in the serous component.

TCGA molecular classification was only possible on Cases M1-M6, where NGS analysis was performed, as the remaining cases had no *POLE* mutational data available. Accordingly, both components of one mixed carcinoma (Case M1) were classified as *POLE*-mutated. Both components of four tumours and one component of one tumour were classified as dMMR The *TP53*-mutated serous carcinoma component of case 5 also showed MMR deficiency, therefore this tumour was classified as dMMR according to the TCGA guidelines and PROMISE algorithm for double classifier tumours [10]. There was only one tumour where there was a difference in the TCGA classification of the two components (Case M2): here, the clear cell carcinoma component was classified as NSMP while the low-grade endometrioid carcinoma as dMMR. No mixed carcinoma was classified as *TP53*-mutated, although no molecular profiling could be performed on Cases M7, M8 and M9, with aberrant p53 expression.

Two of the 5 tumours with dMMR components, where NGS data were available, showed pathogenic mutation in the MMR

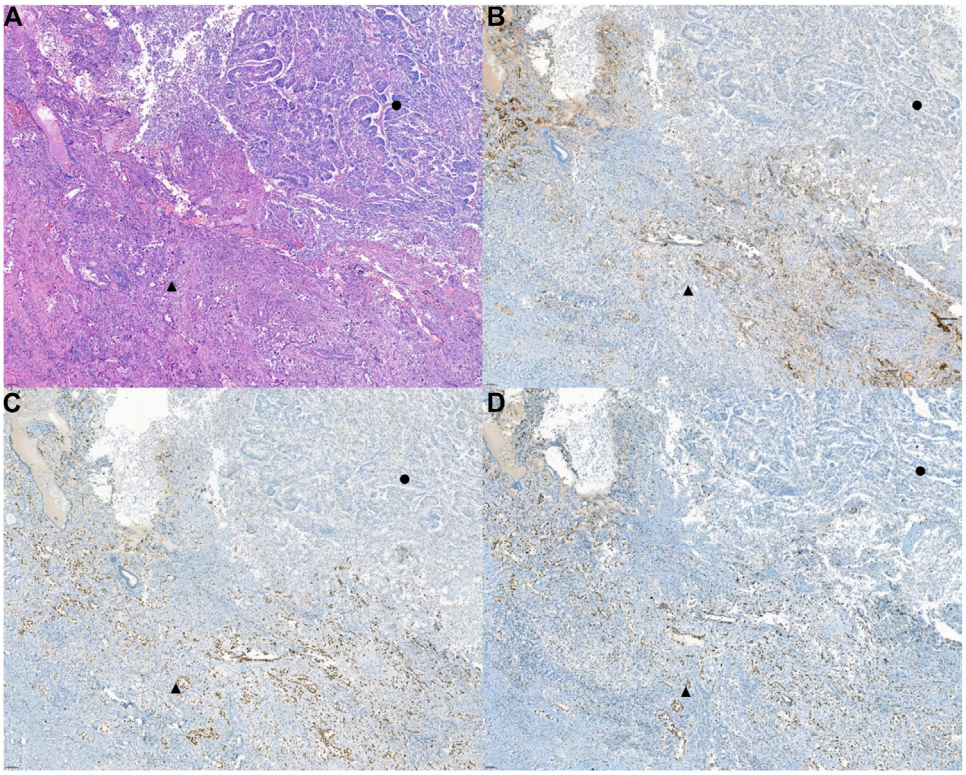


FIGURE 1
Case M2: Mixed low-grade endometrioid and clear cell carcinoma. The black dot and triangle represent the low-grade endometrioid carcinoma component and the clear cell carcinoma component, respectively. **(A)** HE stain (x100). **(B)** NapsinA immunohistochemistry is positive in the clear cell carcinoma and negative in the endometrioid carcinoma component. (x100). **(C)** HNFb immunohistochemistry is positive in the clear cell carcinoma and negative in the endometrioid carcinoma component. (x100). **(D)** PMS2 immunohistochemistry shows loss of expression in the endometrioid carcinoma component, while it is retained in the clear cell carcinoma component. (x100).

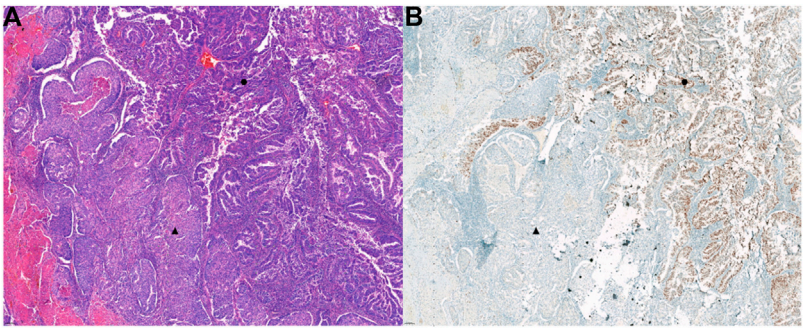


FIGURE 2
Case M5: Mixed serous and high-grade endometrioid carcinoma. The black dot and triangle represent the serous and high-grade endometrioid carcinoma component, respectively. **(A)** HE stain (x100). **(B)** p53 immunohistochemistry shows diffuse nuclear overexpression in the serous carcinoma and wild type staining in the endometrioid carcinoma component. (x100).

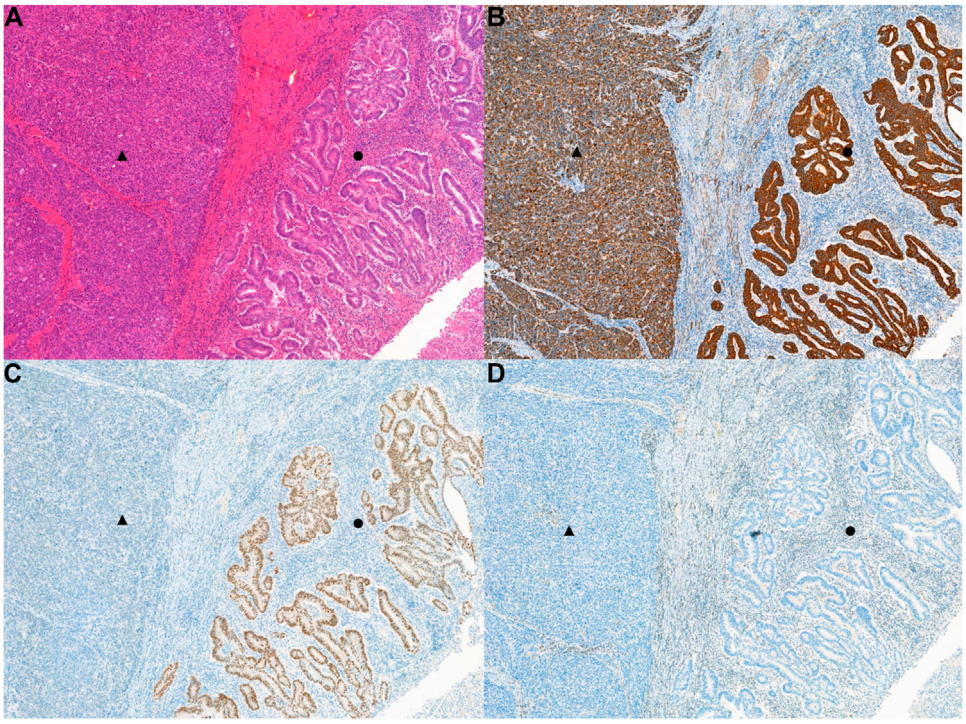


FIGURE 3
Case D1: Dedifferentiated carcinoma with a well-differentiated endometrioid carcinoma and an undifferentiated carcinoma component. The dot and the triangle represent the low-grade endometrioid carcinoma component and the undifferentiated carcinoma component, respectively. **(A)** HE stain (x100). **(B)** CK8-18 immunohistochemistry is positive in both components. (x100). **(C)** PAX8 expression is retained in the endometrioid carcinoma, but lost in the undifferentiated component. (x100). **(D)** PMS2 immunohistochemistry shows loss of expression in both components. (x100).

genes, suggesting that in the other cases loss of protein expression may have occurred due to epigenetic changes, although no methylation analysis for performed to confirm this assumption (Table 2). There were two cases where there was dMMR in both components, but with different patterns. In one case (Case M3), while the low-grade endometrioid carcinoma component showed loss of MLH1 and PMS2 expression, the clear cell carcinoma was MSH6 deficient with a corresponding *MSH6* mutation. In another case (Case M6), one component (high-grade endometrial carcinoma) showed loss of both PMS2 and MLH1 expressions, while the second component (clear cell carcinoma) was only PMS2-deficient on IHC.

In the group of EDCs, all cases underwent NGS analysis and could be classified to TCGA groups (Table 2). The most common differentiated histological type was low-grade

endometrioid carcinoma (7 out of 9 cases) (Figure 3), followed by high-grade endometrioid, and clear cell carcinoma (1 case each). We identified one case with pathogenic *POLE* mutation in both components (Case D8), classified as *POLE*-mutated (1/9). Seven cases had MMR deficiency in both components, classified as dMMR, while one case was dMMR in the undifferentiated component only (Case D2). In this case (Case D2), the low-grade endometrioid component was NSMP. There were two cases with pathogenic *TP53* mutations (Cases D5 and D7), although no aberrant p53 expression was seen in Case D7. These cases were also dMMR on immunohistochemistry, therefore classified as dMMR according to the TCGA guidelines and PROMISE algorithm for double classifier tumours [10]. Out of the 8 cases with dMMR components, only two showed pathogenic MMR gene mutations. Interestingly, in Case D5, while both components showed PMS2 loss on immunohistochemistry, pathogenic *PMS2* mutation was detected only in the low-grade endometrioid carcinoma component. The remaining dMMR cases, where pathoge nic MMR mutation was detected, it is presumed that the loss of MMR protein expression was secondary to promoter methylation, although no methylation studies were performed to substantiate this.

As an increased frequency of protein expression loss of the members of the SWI/SNF pathway was reported in EDCs [11, 12], we specifically looked at the frequency of the mutations of those genes in both tumour groups. In MCEs, pathogenic mutation of *ARID1A* was detected in both components in three cases (3/6). Among the dedifferentiated tumours, six cases had pathogenic mutations of the complex in both components (*ARID1A*), while in one case there was pathogenic mutation (*ARID1A*, Case D9) in the undifferentiated component only (7/9). In one case with identical *ARID1A* mutations in both components, an additional, but different *ARID1A* mutation was detected in both components (Case D8), while in Case D6, the dedifferentiated component showed an additional pathogenic *SMARCA4* mutation.

We also looked at the mutational frequency of genes involved in the PI3K/AKT pathway. All MCEs had either *PIK3CA* or *PTEN* mutations, including three cases with clear cell and three cases with serous carcinoma components. (Cases M3, M4, M6 and Cases M1, M5, M6). This was similar in EDCs, where all cases carried mutations in the PI3K/AKT pathway genes, with *PIK3CA* mutation present in the undifferentiated carcinoma components in 5 cases (Cases D2, D3, D5, D6, and D9) and *PTEN* mutation in 6 cases (Cases D1, D4, D5, D6, D7, and D9).

In terms of genes involved in homologous recombination pathway (HRR), there was *PALB2* mutation detected in both components in one MCE case (Case M5) with *ATM* mutation in the high-grade endometrioid carcinoma component. In two additional cases, *ATM* mutation was detected in one (clear cell carcinoma) component (Cases M3, M6). In EDCs, we found *BRCA2* mutations in both components (Case D8) with an *ATM* mutation in the low-grade endometrioid carcinoma component. This case, however, also carried a pathogenic *POLE* mutation, therefore these HRR gene mutations were likely passenger rather than driver mutations. In Case D7, both components had *ATM* gene mutation, and in Case D9, there was *ATM* mutation in the undifferentiated component only.

Discussion

In this study, we examined MCEs and EDCs in order to assess their genetic composition and investigate their potential pathogenesis. MCEs and EDCs were rare in our cohort, representing less than 1% of the examined tumours (0.63% and 0.57%, respectively). Apart from one MCE, where no mutations were detected in the clear cell component, all MCEs or EDCs examined with NGS had at least one common mutation identified in all components. This supports the hypothesis that both tumour groups either arise through divergent differentiation from a common progenitor or through transdifferentiation [8]. The complete lack of pathogenic mutations in a component of one case was considered more likely to represent an analytical error or could be secondary to low tumour cell content, rather than truly representative of a genetically different phenotype (i.e., collision tumour).

In non-mixed endometrial carcinomas, dMMR tumours were reported to represent about one third of the cases [6, 7]. In our cohort, there was a considerably higher frequency of dMMR in both MCEs and EDCs (60% and 83%, respectively). Both groups also contained *POLE*-mutated phenotypes, while *TP53*-mutated and NSMP tumours were rare. High frequency of dMMR has been reported in EDC [6, 7, 13, 14], while there are reports of frequent dMMR in mixed clear cell/endometrioid carcinomas, but not in mixed serous/endometrioid carcinomas [7, 15]. Our results in MCEs may therefore be biased by the high proportion of clear cell/endometrioid tumours in our cohort (6 cases), out of which 5 were dMMR in at least one component, while the sixth case was *POLE*-mutated one. There was a relatively low number of serous/endometrial carcinomas in our set (3 cases), out of which 1 was dMMR. The remaining case, a mixed serous/clear cell carcinoma did not show MMR deficiency or *POLE* mutation. Nevertheless, the high proportion of tumours with DNA repair deficiency (mainly dMMR but also with *POLE* mutation) suggest that genetic heterogeneity is likely driven by genomic hypermutability in both MEC (at least those with clear cell carcinoma component) and EDC.

A recent Japanese study suggested a potential role of HRR gene mutation in mixed endometrial tumours, with a frequency of 30%, and a potential therapeutic role in PARP inhibitor therapy [16]. The high proportion of MMR (and *POLE*) mutation and genomic hypermutability among both MECs and EDCs, however, may introduce potential difficulties in any further analysis of the genotype of these tumours. As these tumours are characterised by high tumour mutational burden due to an erroneous DNA repair mechanism, it is difficult to assess the significance of a pathogenic mutation with regards to their driver or passenger status in tumour pathogenesis. This was also taken into consideration in the TCGA molecular profiling, where *POLE*-mutated and dMMR phenotypes take precedence over *TP53* mutation in endometrial carcinomas. Nevertheless, we found a similar frequency (30%) of HRR gene

mutation in our cohort. There were HRR gene mutations detected in 2 (out of 6) MCEs and 3 (out of 9) EDCs - one of the latter being also *POLE*-mutated. The clinical and therapeutic significance of these findings however, are uncertain, as to the best of our knowledge there are no reports of high incidence of HHR deficiency/high genomic instability score in endometrial carcinoma. Furthermore, trials of additional PARP inhibitor therapy in endometrial carcinomas appear more empirical rather than based on the tumours' HRR status [17].

Loss of *ARID1A* (part of the SWI/SNF complex) expression is considered a poor prognostic factor in endometrial carcinoma, and mutation of other genes of the SWI/SNF complex have been reported at a higher rate in dedifferentiated and undifferentiated endometrial carcinomas [13, 14]. The SWI/SNF complex plays a role in chromatin remodelling, and disruption of this pathway affects homeostasis and cellular development [18]. Alterations of this pathway is associated with a poorer prognosis in endometrial carcinoma, with rapid disease progression, and therapy resistance even when EDCs are considered separately [11, 12]. Mutation of the SWI/SNF complex (predominantly *ARID1A*) occurs in up to 40% in non-mixed endometrioid carcinoma, and 25% in clear cell carcinoma of the uterus [19]. However, this genetic change is rare in serous endometrial carcinoma, with mutations in both *ARID1A* and *TP53* considered mutually exclusive [18]. This reported high frequency of SWI/SNF mutations in both undifferentiated and dedifferentiated endometrial carcinomas may suggest that in EDCs, it is the undifferentiated component that carries the mutation. In line with previous reports, there was a high frequency of mutation (78%, 7 out 9) of the SWI/SNF complex in EDCs (mainly *ARID1A* mutation, with *SMARCA4* mutation in one case) in our cohort. However, out of these 7 cases, *ARID1A* mutation was present not only in the undifferentiated, but also in the other, differentiated carcinomatous component. This suggests that *ARID1A* mutation is a relatively early event in tumorigenesis, occurring before the transdifferentiation or divergent differentiation of the tumours, rather than a new event in the undifferentiated component. In MCEs, only *ARID1A* mutation was detected, but again, it was present in both components of 50% in the MCEs (3 out of 6, all mixed clear cell/endometrioid).

Similar to the SWI/SNF pathway, mutations and disruption of the PI3K/PTEN pathway, mainly *PTEN* and *PIK3CA* mutations, are predominantly involved in the pathogenesis of endometrioid endometrial carcinomas (up to 50%), with frequent co-occurence of *PIK3CA* and *PTEN* mutations [20]. Activation of this pathway affects gene expressions that regulate apoptosis and promote cell proliferation. In pure clear cell or serous carcinoma, *PIK3CA* mutation is less frequent (<30%), while *PTEN* mutation is exceedingly rare (0%–15%) [15, 21]. In our cohort, all MCEs and EDCs showed mutation in *PIK3CA* and/or *PTEN* genes in at least one component. In addition, in contrast to pure serous and clear carcinomas, it was frequent in serous and clear cell carcinomatous components of MCEs (4/6). This again suggests that the pathogenesis of these tumours differs from pure serous and clear cell carcinomas, supporting the hypothesis of transdifferentiation or divergent differentiation from an endometrioid carcinomatous component, with *PIK3CA* and *PTEN* mutation being an early event in carcinogenesis.

MCEs and EDCs are rare, and this is the cause of our relatively low case number, which is the main limitation of our study. Our results, however still suggest that the pathomechanisms of MCEs and EDCs are very similar in many aspects: they both appear to arise predominantly on a background of dMMR and genomic hypermutability, by means of transdifferentiation or divergent differentiation. Nevertheless EDCs show a higher frequency of SWI/SNF mutations and according to previous studies, still carry a poorer prognosis. [2, 22, 23]. Although our study lacks clinical follow-up data, due to this disparity in involvement of the SWI/SNF complex and the reported difference in prognosis observed in EDC and MCE, it may be worthwhile to maintain a separate WHO classification for endometrial carcinomas, despite their shared pathogenesis and clonal origin [1].

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by Medical Research Council - Scientific and Research Ethics Committee, Health and Scientific Committee of the Ministry of Human Resources of Hungary (approval number: BM-794-1-2024). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

JB: case review, writing – original draft, research and data collection; IV: conceptualization, writing – review and editing LS: research and data collection; AS: research and data collection; ET: resources, supervision; LK: supervision, writing – review and editing; ZM: conceptualization, writing – original draft. All authors contributed to the article and approved the submitted version.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This research was funded by

HUN-REN-ONKOL-TTK-HCEMM Oncogenomics Research Group.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.por-journal.com/articles/10.3389/pore.2026.1612616/full#supplementary-material

SUPPLEMENTARY TABLE S1
Antibody clones and dilutions used for immunohistochemistry.

SUPPLEMENTARY TABLE S2
Pathogenic point mutations detected in each component of mixed endometrial carcinomas and endometrial dedifferentiated carcinomas. Abbreviations: clear cell carcinoma (CCC), low grade endometrioid carcinoma (LGEC), SC (serous carcinoma), high grade serous carcinoma (HGEC), undifferentiated carcinoma (UC).

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