



Molecular Evaluation of Endometrial Dedifferentiated Carcinoma, Endometrioid Carcinoma, Carcinosarcoma, and Serous Carcinoma Using a Custom-Made Small Cancer Panel

Yusuke Kobayashi^{1†}, Ikumi Kitazono^{2†}, Toshiaki Akahane^{2,3}, Shintaro Yanazume⁴, Masaki Kamio⁴, Shinichi Togami⁴, Sachio Nohara⁵, Ippei Sakamoto⁵, Seiya Yokoyama², Kazuhiro Tabata², Hiroaki Kobayashi^{1,4} and Akihide Tanimoto^{2,3*}

¹Course of Advanced Cancer Medicine for Gynecologic Cancer, Kagoshima, Japan, ²Department of Pathology, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan, ³Center for Human Genome and Gene Analysis, Kagoshima University Hospital, Kagoshima, Japan, ⁴Department of Obstetrics and Gynecology, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan, ⁵Department of Biomedical Informatics, Mitsubishi Space Software, Amagasaki, Japan

OPEN ACCESS

Edited by:

Anna Sebestyén,
Semmelweis University, Hungary

*Correspondence:

Akihide Tanimoto
akit09@m3.kufm.kagoshima-u.ac.jp
orcid.org/0000-0001-7508-1624

[†]These authors have contributed
equally to this work

Received: 06 August 2021

Accepted: 28 October 2021

Published: 23 December 2021

Citation:

Kobayashi Y, Kitazono I, Akahane T, Yanazume S, Kamio M, Togami S, Nohara S, Sakamoto I, Yokoyama S, Tabata K, Kobayashi H and Tanimoto A (2021) Molecular Evaluation of Endometrial Dedifferentiated Carcinoma, Endometrioid Carcinoma, Carcinosarcoma, and Serous Carcinoma Using a Custom-Made Small Cancer Panel. *Pathol. Oncol. Res.* 27:1610013. doi: 10.3389/pore.2021.1610013

It is often difficult to histologically differentiate among endometrial dedifferentiated carcinoma (DC), endometrioid carcinoma (EC), serous carcinoma (SC), and carcinosarcoma (CS) due to the presence of solid components. In this study, we aimed to categorize these carcinomas according to The Cancer Genome Atlas (TCGA) classification using a small custom-made cancer genome panel (56 genes and 17 microsatellite regions) for integrated molecular diagnosis. A total of 36 endometrial cancer cases with solid components were assessed using IHC, next-generation sequencing (NGS), and the custom-made panel. Among 19 EC cases, six were categorized as MMR-deficient (MMR-d) and eight were classified as having a nonspecific molecular profile. Three EC cases were classified as *POLE* mutation (*POLEmut*)-type, which had a very high tumor mutation burden (TMB) and low microsatellite instability (MSI). Increased TMB and MSI were observed in all three DC cases, classified as MMR-d with mutations in *MLH1* and *POLD1*. Except for one case classified as MMR-d, all SC cases exhibited *TP53* mutations and were classified as p53 mutation-type. SC cases also exhibited amplification of *CCND1*, *CCNE1*, and *MYC*. CS cases were classified as three TCGA types other than the *POLEmut*-type. The IHC results for p53 and ARID1A were almost consistent with their mutation status. NGS analysis using a small panel enables categorization of endometrial cancers with solid proliferation according to TCGA classification. As TCGA molecular classification does not consider histological findings, an integrated analytical procedure including IHC and NGS may be a practical diagnostic tool for endometrial cancers.

Keywords: microsatellite instability, tumor mutation burden, integrated molecular diagnosis, endometrial cancers, solid proliferation

INTRODUCTION

Dedifferentiated carcinoma (DC) is a rare endometrial cancer accounting for 2% of all endometrial cancers. It is composed of well differentiated endometrioid carcinoma (EC) and undifferentiated carcinoma [1]. Contrastingly, carcinosarcoma (CS) is composed of high-grade carcinomatous and sarcomatous components, with the latter containing homologous and/or heterologous elements [1]. Identifying heterologous elements via immunohistochemistry (IHC) is helpful for differentially diagnosing DC and CS cases. However, in some cases, both the dedifferentiated part of DC and the sarcomatous part of CS exhibit similar nonspecific vimentin and keratin expression [2]. Grade 2 (G2) and Grade 3 (G3) EC cases also exhibit variable vimentin and keratin expression [3], indicating that the value of IHC for vimentin and keratin is limited in endometrial cancer diagnosis. Therefore, developing a novel integrated strategy combining histological and genomic analyses is necessary for the differential diagnosis of DC, EC, serous carcinoma (SC), and CS with areas of solid proliferation [4-6].

Next-generation sequencing (NGS) has recently become a standard procedure for cancer genomic analysis [7, 8]. This is largely due to the development of improved techniques, which allow the use of formalin-fixed paraffin-embedded (FFPE) tissues and liquid-based cytology specimens [9, 10]. We previously established a cancer gene panel comprising 60 genes and 17 microsatellite foci. This customized panel was used to analyze genetic profiles using FFPE tissues of endometrial cancers in terms of gene mutations, tumor mutation burden (TMB), and microsatellite instability (MSI) [11]. In this study, the above panel was further modified to detect *POLE* for evaluating gene alterations that can categorize DC, CS, SC, and EC with solid proliferation into MMR-deficient (MMR-d), p53 mutation (p53mut)-type, *POLE* mutation (*POLE*mut)-type, and cases with no specific molecular profile (NSMP) according to The Cancer Genome Atlas (TCGA) classification. Furthermore, we used an IHC panel containing mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, and MSH6), p53, ARID1A, PTEN, vimentin, WT-1, estrogen receptor (ER), and cyclin D1 (*CycD1*), which is routinely available for FFPE tissue sections in pathology laboratories. This panel was used to determine whether an integrative approach utilizing IHC and NGS could aid pathologists in the differential diagnosis and histology-dependent classification of endometrial cancers.

MATERIALS AND METHODS

Ethics Approval and Samples

All patients were registered at the Clinical Research of Cancer Gene Panel Analysis of Gynecologic Cancers Study, which was conducted from January 2019 to October 2021 at the Kagoshima University Hospital. The clinical samples used in this study were approved by the Ethics Committees for Clinical and Epidemiologic Research at Kagoshima University (approval number: 180215) and written informed consent was obtained from all participants. Among the 155 cases entered, 36 cases, including 16 G2 and 3 G3 EC cases, 3 DC cases, 4 CS cases, 9 SC cases, and 1 large cell neuroendocrine carcinoma (LCNEC) case with areas of solid proliferation were

included in this study. Clear cell carcinoma (CCC) is considerably less frequent in the Gynecologic Cancers Study in our hospital, therefore, CCC cases were excluded from this study. No patients with undifferentiated carcinoma were included.

Tissue Preparation and Diagnostic Criteria

Resected tissues obtained by hysterectomy were fixed with 10% neutral phosphate-buffered formalin, routinely processed for paraffin embedding, and sectioned for hematoxylin and eosin (HE) staining, IHC, and NGS. Pathological diagnoses were made according to the World Health Organization (WHO) classification system [1]. The following criteria were used for diagnosing SC: carcinoma showing complex papillary, glandular and/or solid growth patterns with marked nuclear pleomorphism; CS: admixture of high-grade müllerian type carcinomatous elements and apparent mesenchymal morphology, as determined using homologous (CD10, desmin, alpha-smooth muscle actin) or heterologous (myogenin, MyoD1, S-100) IHC markers [12, 13]; DC: biphasic tumor consisting of EC and undifferentiated nested or trabecular architecture with no gland formation; EC: glandular proliferation with smooth luminal outline, composed of columnar cells with pseudostratified nuclei. Grading criteria of EC: Grade 1, 2, and 3, respectively, exhibit $\leq 5\%$, 6–50%, and $>50\%$ solid growth was used.

The dedifferentiated parts of DC are positive for epithelial marker expression in scattered cells, and mesenchymal elements of CS are weakly positive or negative for these markers; hence, diffuse expression of keratins (AE1/AE3 and CAM5.2) and EMA was used to differentiate endometrial cancers [1].

Criteria for Evaluating the IHC Results of MMR Proteins, p53, ARID1A, and PTEN

The antibodies used for IHC are listed in Online Resource 1. MMR-proficient (MMR-p) was defined as positive nuclear staining for all MMR proteins (MLH1, PMS2, MSH2, and MSH6). MMR-d was defined as the complete absence of nuclear staining for any MMR proteins [14]. p53 expression resulting in scattered nuclear staining with variable intensity was categorized as wildtype (wt p53) pattern, whereas diffuse and strong nuclear overexpression or a complete loss of expression was defined as mutation (mt p53) pattern. For ER, vimentin, WT-1, *CycD1*, ARID1A, and PTEN expression, the percentage of marker-positive areas assessed in the tumor section were evaluated in 10% increments. In areas of glandular or carcinomatous and solid proliferation, the expression of ARID1A and PTEN was categorized as very low at $<10\%$, and as lost at $<5\%$.

Cancer Panel Design, DNA Isolation, and NGS Analysis

A cancer panel was redesigned by making a minor modification of the previous panel [11] to include 56 cancer-related genes and 17 microsatellite foci selected from the QIAseq Targeted DNA Custom Panel (Qiagen, Reston, VA, United States), with 2,640 primers for the regions of interest (194,131 bp) and an average exon coverage of 99.87% (Online Resource 2). Whole blood DNA was extracted using QIAamp DNA Blood Mini Kits (Qiagen). Cancer DNA was obtained from three to six sections (10 μm

thickness) of FFPE tissues, representing more than 30% of the cancerous tissue. When cancer is composed of various histology, several tissue sections were subjected to NGS analysis if separate sampling of each element was possible by micro or macrodissection. The FFPE sections were first incubated with proteinase K (Promega, Madison, WI, United States) for 15 h at 70°C, followed by incubation at 98°C for 1 h in the lysis buffer (Promega). Following centrifugation (12,000 × g, 5 min at 4°C), the supernatants were applied to a Maxwell[®] RSC DNA FFPE kit and the Maxwell 16 system (Promega). The concentrations of extracted DNA were measured using Qubit 3.0 dsDNA BR assay kits (Life Technologies, Grand Island, NY, United States), and the DNA quality was monitored using the QIAseq DNA quantiMIZE kit (Qiagen). DNA with a quality check score <0.04 was considered high-quality DNA [11]. NGS was performed using a MiSeq sequencer (Illumina, San Diego, CA, United States) as previously described [11].

Sequence data were annotated as previously described using the Qiagen Web Portal service (<https://www.qiagen.com/us/shop/genes-and-pathways/data-analysis-center-overview-page/>) and Mitsubishi Space Software (Amagasaki, Hyogo, Japan) [11, 15]. The COSMIC database and human genome reference GRCh37 (hg19) (https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.13/) were used as references. The sequence data obtained from whole blood DNA were used only as a reference, and germline analysis was not performed.

Calculation of Copy Number Alteration

To calculate the copy number (CN) from the baseline data used for counting correction per amplicon, the number of reads sequenced in each amplicon was counted, and the reads per million (RPM) value was determined. The RPM coefficient of variation (CV), mean, and median value per amplicon in at least 100 FFPE samples were calculated. The RPM median of the amplicons with a CV < 0.34 and mean > 10 was set as the baseline. The number of reads sequenced in each 56-panel amplicon was counted in the sample to calculate the CN of each sample. The baseline ratios {log₂ ratio [= log₂ (sample RPM/baseline RPM median)]} in amplicons that satisfied the conditions of CV < 0.34 and mean > 10 were counted, and the overall SD and median value of the log₂ ratio for each gene was calculated. The genes with a log₂ ratio median value > 2 SD were categorized as amplified, while the genes with a log₂ ratio median value < -2 SD were categorized as gene loss [16].

Calculation of Cut-Off Values for TMB and MSI Scores

Missense mutations with more than 10% variant allele frequency, including nonsynonymous mutations and internal deletions, were counted as somatic mutations. The TMB was calculated as the number of single nucleotide variants million per base pairs (Mbp) of the DNA sequence [17, 18], and the MSI scores were determined using MSIsensor (ver. 1.0) [19, 20]. To determine the cutoff values of MSI and TMB using receiver operator characteristic curves, 59 samples from 41 endometrial cancer cases were used.

Statistical Analysis

All values were expressed as the mean ± standard error. Significant differences were identified using student's or Welch's *t*-tests and were considered significant at *p* < 0.05.

RESULTS

IHC Profiles

The IHC results regarding the MMR proteins, p53, ARID1A, PTEN, WT-1, ER, CycD1, and vimentin are summarized in **Table 1** (extracted) and Online Resource 3 (detailed), and representative photomicrographs are shown in **Figure 1**. Among 19 EC cases, 16 showed the wt p53 pattern, and three showed the mt p53 pattern. Six EC and three DC cases showed loss of MMR protein expression. PTEN expression was very low (<10% area showing expression) or lost (<5% area showing expression) in all DC (3/3) and most EC cases (12/19), while ARID1A was lost less frequently in DC (1/3) and EC cases (7/19). All SC (9/9) and some CS (2/4) cases exhibited the mt p53 pattern in carcinomatous and sarcomatous elements. Three CS cases were MMR-p, whereas one was MMR-d. Almost all SC cases were MMR-p (8/9). PTEN expression was lost in one CS and five SC cases. ARID1A expression was well preserved in CS (3/4) and SC (8/9) cases. One LCNEC exhibited MMR-p and had mt p53 as well as diffuse PTEN and ARID1A expression. The expression of WT-1, vimentin, CycD1, and ER was variable in all carcinoma types. The genomic correlation between CycD1, ER, PTEN, and ARID1A is described later.

Cutoff Values of TMB and MSI Scores

The TMB values of wildtype and mutated *POLE* cases were 36.3 ± 3.8 and 175.1 ± 61.0 (*p* = 0.026), respectively, and the cutoff value for TMB-ultrahigh (TMB-UH) was calculated as 72. Excluding the *POLE*mut cases, the TMB values of MMR-p and MMR-d cases were 22.5 ± 3.0 and 63.0 ± 5.1, respectively (*p* < 0.001), and the cutoff values for TMB-high (TMB-H) and -low (TMB-L) were calculated to be 42. Similarly, the MSI values of MMR-p and MMR-d cases were 3.6 ± 0.5 and 38.7 ± 2.0, respectively (*p* < 0.001), and the cutoff values for the differentiation of MSI-high (MSI-H) and -low (MSI-L) were estimated to be 13. The distribution of TMB and MSI scores of the tested endometrial cancers for cutoff value estimation is shown in **Figure 2**.

Genomic Profile

Genomic profiles of 36 cases are shown in **Table 1** (extracted) and Online Resource 4 (detailed). All three DC cases (no. 20–22) carried *MLH1* and *POLD1* mutations with TMB-H and MSI-H, and exhibited variable mutations in *PTEN*, *ARID1A*, *PIK3CA*, *PIK3R1*, and *CTNNB1*. The *TERT* coding region was mutated in two DC cases, although the promoter was not. All 19 EC cases exhibited common mutations in *PTEN*, *ARID1A*, *CTNNB1*, *PIK3CA*, and *PIK3R1*. Among the 19 cases, 16 cases (except for case no. 2, 13, and 19) included MMR-p and MMR-d cases and exhibited higher TMB scores than did the SC cases (**Table 2**). Six EC cases that were MMR-d had higher TMB and MSI scores than the ten MMR-p cases and were classified as TMB-H and MSI-H

TABLE 1 | Summary for histological diagnosis, expression of MMR proteins and p53, and genomic profile in 36 cases.

Case no.	Age	Histological diagnosis	MMR	p53	MSI	TMB	SNV and delins mutations							TCGA type				
							TP53	MLH1	PMS2	MSH2	MSH6	POLE	POLD1		TERT			
1	60	EC (G2)	pro.	wt	Low	High									NSMP			
2	54	EC (G1)	pro.	wt	Low	U-high			p.Val63Met		c.2389G>T	p.Val411Leu			POLEmut			
		EC (G2)			Low	U-high					c.2389G>T	p.Val411Leu						
3	67	EC (G2)	pro.	wt	Low	Low									NSMP			
4	68	EC (G2)	def.	wt	High	High									MMR-d			
5	63	EC (G2)	pro.	wt	Low	Low									NSMP			
6	64	EC (G3)	def.	wt	High	High	p.Arg202Cys								MMR-d			
7	59	EC (G2)	def.	wt	High	High					p.Pro991Leu				MMR-d			
8	55	EC (G2)	pro.	wt	Low	Low									NSMP			
9	60	EC (G2)	def.	wt	High	High				p.Phe22fs					MMR-d			
10	61	EC (G2)	pro.	wt	Low	Low									NSMP			
11	78	EC (G3)	def.	wt	High	U-high	p.Arg175His					p.Met491fs			MMR-d			
12	60	EC (G2)	pro.	wt	Low	Low									NSMP			
13	55	EC (G2)	pro.	mt	Low	U-high	p.Arg213*		p.Arg107Trp p.Arg134Gln p.Arg427Cys	p.Asp758Asn	p.Arg298Gln p.Lys632Arg p.Glu641* p.Glu995Lys	p.Pro286Ser p.Val411Leu		p.Ser579Asn c.*334C>T	POLEmut			
14	65	EC (G2)	gld.	pro.	mt	Low	Low	p.Arg273Cys										
			solid	pro.	mt	Low	Low											
15	68	EC (G2)	gld.	pro.	wt	Low	Low											
			solid	pro.	wt	Low	High			c.903+2T>A							NSMP	
16	55	EC (G3)	pro.	mt	Low	Low	c.993+1G>T									p53mut		
17	73	EC (G2)	gld.	pro.	wt	Low	Low											
			solid	pro.	wt	Low	Low										NSMP	
18	53	EC (G2)	gld.	def.	wt	High	Low											
			solid	def.	wt	High	U-high			p.Glu613_Phe614del							MMR-d	
19	56	EC (G2)	gld.	pro.	wt	Low	U-high											
			solid	pro.	wt	Low	U-high			p.Glu483*	p.Arg1201Gln p.Glu1234*	p.Pro286Arg					POLEmut	
20	51	DC	EC (G1)	def.	wt	High	High											
			De			High	High			p.Ser388Phe				p.Asp289dup				MMR-d
21	57	DC	EC (G3)	def.	wt	High	High	p.Lys373fs										
			EC (G1)			High	High			p.Asn287fs								MMR-d
			De			High	High	p.Lys373fs					p.Ala69Thr		p.Tyr581His	p.Trp371Cys		
22	71	DC	EC (G1)	def.	wt	High	High											
			De			High	High			p.Ser577Ser p.Trp712*								
23	64	SC	pro.	mt	Low	Low	p.Arg249Ser									p53mut		
24	67	SC	pro.	mt	Low	Low	p.Gly245Ser p.Leu43fs									p53mut		
25	57	SC	pro.	mt	Low	Low	p.Arg248Gln									p53mut		
26	63	SC	Ad	pro.	mt	Low	Low	p.Ile255Ser										
			Spn			Low	Low	p.Ile255Ser									p53mut	

(Continued on following page)

TABLE 1 | (Continued) Summary for histological diagnosis, expression of MMR proteins and p53, and genomic profile in 36 cases.

Case no.	Age	Histological diagnosis	MMR	p53	MSI	TMB	SNV and delins mutations						TCGA type	
							TP53	MLH1	PMS2	MSH2	MSH6	POLE		POLD1
27	72	SC	def.	mt	High	U-high	p.Arg306*						p.Arg352His c.2466+4C>T	p53mut/ MMR-d
28	75	SC	pro.	mt	Low	Low	p.Ser241Phe							p53mut
29	70	SC	pro.	mt	Low	Low	p.Gln144*							p53mut
30	65	SC	pro.	mt	Low	Low	c.817C>T							p53mut
31	77	SC	pro.	mt	Low	Low	p.Ala276Gly							p53mut
32	80	CS	pro.	mt	Low	Low	p.Asn239Ser							p53mut
33	58	CS	pro.	mt	Low	Low	p.Cys176Phe c.706T>A							p53mut
34	63	CS	Car	pro.	wt	Low	Low							NSMP
			Src											
35	64	CS	Car	def.	wt	High	High							MMR-d
			Src											
36	63	LCNEC	pro.	mt	Low	Low	p.Phe113Ser							p53mut

EC, endometrioid carcinoma; gland., glandular part; solid, solid part; DC, dedifferentiated endometrioid carcinoma; CS, carcinosarcoma; SC, serous carcinoma; LCNEC, large cell neuroendocrine carcinoma; De, dedifferentiated part; Ad, adenocarcinoma part; Car, carcinomatous part; Sar, sarcomatous part; Spn, spindle cell part; MMR, mismatch repair protein; MSI, microsatellite instability; TMB, tumor mutation burden; CNA, copy number alteration; Amp, amplification; SNV, single nucleotide variant; U-high, ultra high; pro., proficient; def., deficient; wt, wild-type; mt, mutation; PLOEmut, POLE, mutation; MMR-d, MMR-deficient; NSMP, no specific molecular profile; p53mut, p53 mutation.

(Table 3). Three EC cases (no. 2, 13, and 19) that harbored a *POLE* mutation were classified as TMB-UH and MSI-L.

Among the four CS cases (no. 32–35), two contained mutations in *TP53* and exhibited mt p53 expression. Furthermore, three CS cases, one with mutated and two with wildtype *TP53*, exhibited amplification of *MYC*, *CCNE1*, or *CCND1*. All CS cases were MMR-p (MSI-L and TMB-L) and showed no mutations in *MMR*, except for one case that exhibited a loss of *MLH1* and *PMS2* expression (MSI-H and TMB-H) without any mutations in *MLH1* and *PMS2*. IHC and NGS profiles of eight SC cases, displaying mt p53 expression and MMR-p characteristics were classified as MSI-L and TMB-L. One CS case (no. 32) showed *ERBB2* amplification. Among the eight SC cases, five showed amplification in *MYC*, *CCNE1*, or *CCND1*. One SC case (no. 27) exhibited MMR-d with wildtype *MMR* and was classified as TMB-UH and MSI-H. LCNEC (no. 36) exhibited an SC-like profile with mutations in *TP53* and *PIK3CA*. Other mutations such as *BRCA1*, *BRCA2*, and *ATM* were detected in some cases (nos. 2, 4, 5, 18, 19, 20, 22, 27, and 36) (Online Resource 4).

For the tumors composing of heterogenous elements, the genomic profile was evaluated separately for each element suing two or three FFPE sections in six cases of EC (no. 2, 14, 15, and 17–19), two of CS (nos. 34 and 35), one of SC (no. 26), and three of DC (nos. 20, 21, and 22). The genomic profiles were not exactly matched between the heterogenous elements, but were not so different as much as TCGA classification was revised (Online Resource 4).

Correlation Between the Results of Genomic and IHC Analyses of p53, ARID1A, and PTEN

The correlation between the genomic and IHC analyses of ARID1A and PTEN is presented in Online Resource 5. Most

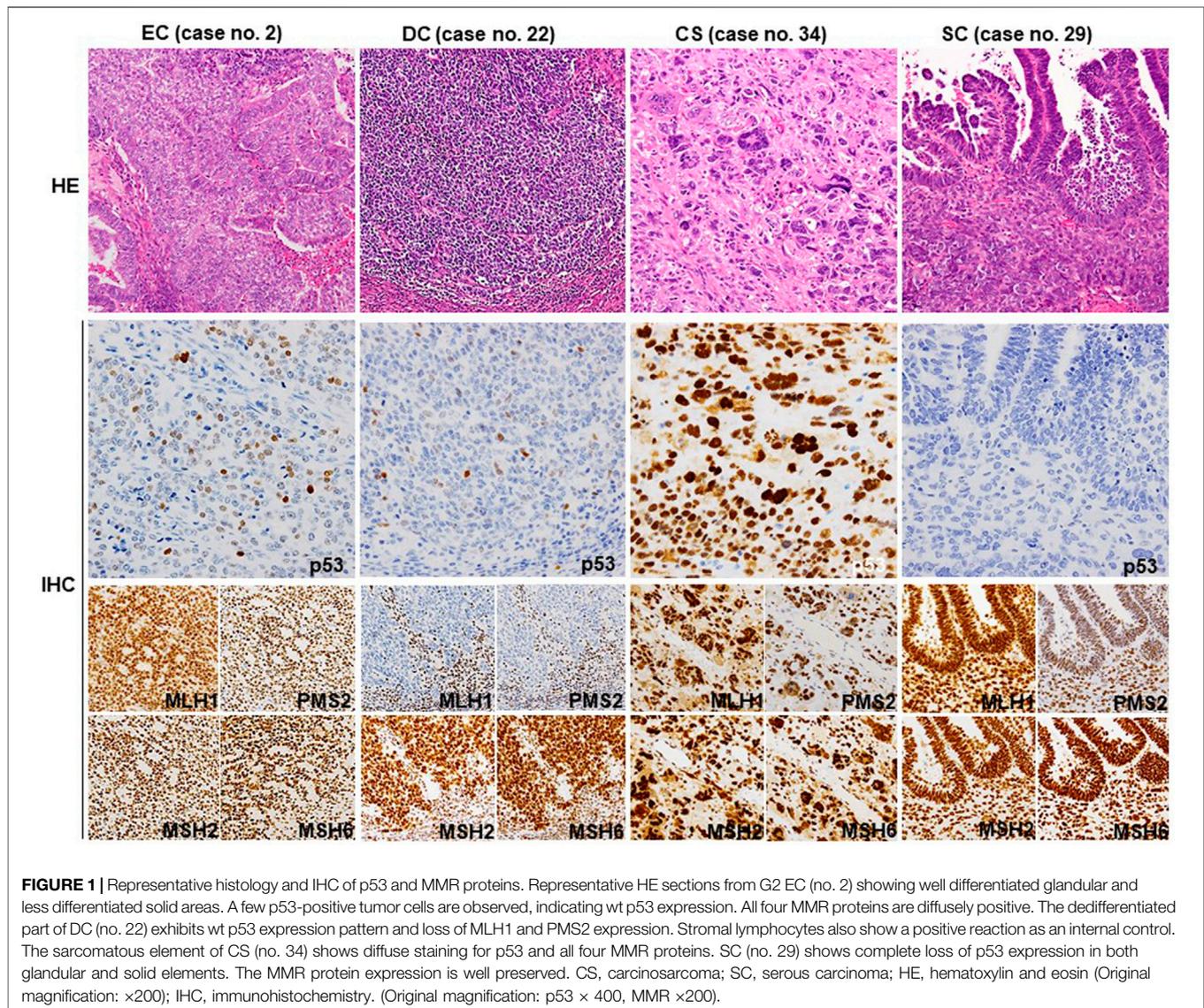
CS (3/4) and SC cases (8/9) exhibiting high ARID1A expression (>90% of the positive area) had no mutations. Fourteen EC cases (14/19) harbored frameshift, nonsense mutations or splice variants. Among these, eight cases exhibited a loss of ARID1A expression (<5% of the positive area). PTEN expression was lost in one CS case with gene alterations (1/4). Although SC cases did not display *PTEN* mutations (9/9), five cases did not express PTEN. Among 19 EC cases, 18 carried *PTEN* mutations and 13 accompanied by a loss of PTEN expression (<5% of the positive area). As previously reported [21, 22], ARID1A expression was almost consistent with *ARID1A* mutations in EC, CS, and SC cases, and that of PTEN was consistent with *PTEN* mutations in EC cases. For CycD1 and ER, a correlation between genomic and IHC analysis results could not be established due to the limited number of cases exhibiting these gene mutations (data not shown). The status of *TP53* mutation matched well with the p53 IHC results in EC, SC, CS, and LCNEC cases (33/36), except for two EC cases (no. 6 and 11) and one DC case (no.21) that exhibited wt p53 IHC but harbored *TP53* mutations.

TCGA Classification

Based on TCGA classification [23], EC cases were classified as *POLE*mut (3/19), MMR-d (6/19), NSMP (8/19), and p53mut (2/19), and all DC cases were categorized as MMR-d (3/3). CS cases were classified as MMR-d (1/4), p53mut (2/4), and NSMP (1/4). Most SC cases were classified as p53mut (8/9), and one was classified as MMR-d (1/9) (Table 4).

DISCUSSION

Our study indicated that NGS-based genomic analysis using the custom-made small panel could be used to evaluate TMB and



MSI and for the detection of gene mutations, thus aiding in the categorization of endometrial cancers with solid proliferation according to TCGA classification.

DC comprises well differentiated EC and undifferentiated carcinoma. Therefore, it is often difficult to differentiate DC from CS and EC. Differential diagnosis by HE staining alone is challenging when CS tissues exhibiting only subtle spindle cells or ambiguous heterologous sarcomatous elements, such as chondrosarcoma and rhabdomyosarcoma, and less evident serous morphology are involved [24]. Differentiation markers, such as myogenin, CD10, and desmin, are used to investigate the presence of heterologous or homologous sarcomatous elements in CS tissues. However, sarcomatous markers may be expressed focally even in carcinomatous areas [2, 3]. Moreover, the epithelial markers (keratins and EMA) were sometimes weakly expressed in the scattered cells of dedifferentiated elements of DC or absent in the sarcomatous parts of CS tissues [1]. Therefore, it may be difficult to differentiate between DC and CS, even with additional

analysis using IHC. The effects of interobserver variability on the diagnosis of DC, CS, and EC are well established [25, 26]. Therefore, IHC results alone may be insufficient for distinguishing these cancers. Consequently, genomic analysis appears to be a more suitable option for developing an integrated strategy for the differential diagnosis of endometrial cancers [27-29].

We found that DC cases typically harbored mutations in *MLH1* and *POLD1* as well as exhibited variable mutations in *PTEN*, *ARID1A*, *PIK3CA*, *PIK3R1*, and *CTNNB1* with MSI-H and TMB-H. Contrastingly, most SC cases and some CS cases (MSI-L and TMB-L) carried *TP53* mutations, while *PTEN*, *ARID1A*, *PIK3CA*, *PIK3R1*, and *CTNNB1* mutations were less frequent. Since CS is a biphasic tumor with a carcinomatous element exhibiting high-grade müllerian type carcinoma [12, 13, 24], CS cases harbored a *TP53* mutation, similar to SC [30, 31]. MMR-d cases, which are mutually exclusive of *TP53* mutations [32, 33], are rarely observed in CS or SC (4-6%) [24, 30, 34-36]. As previously

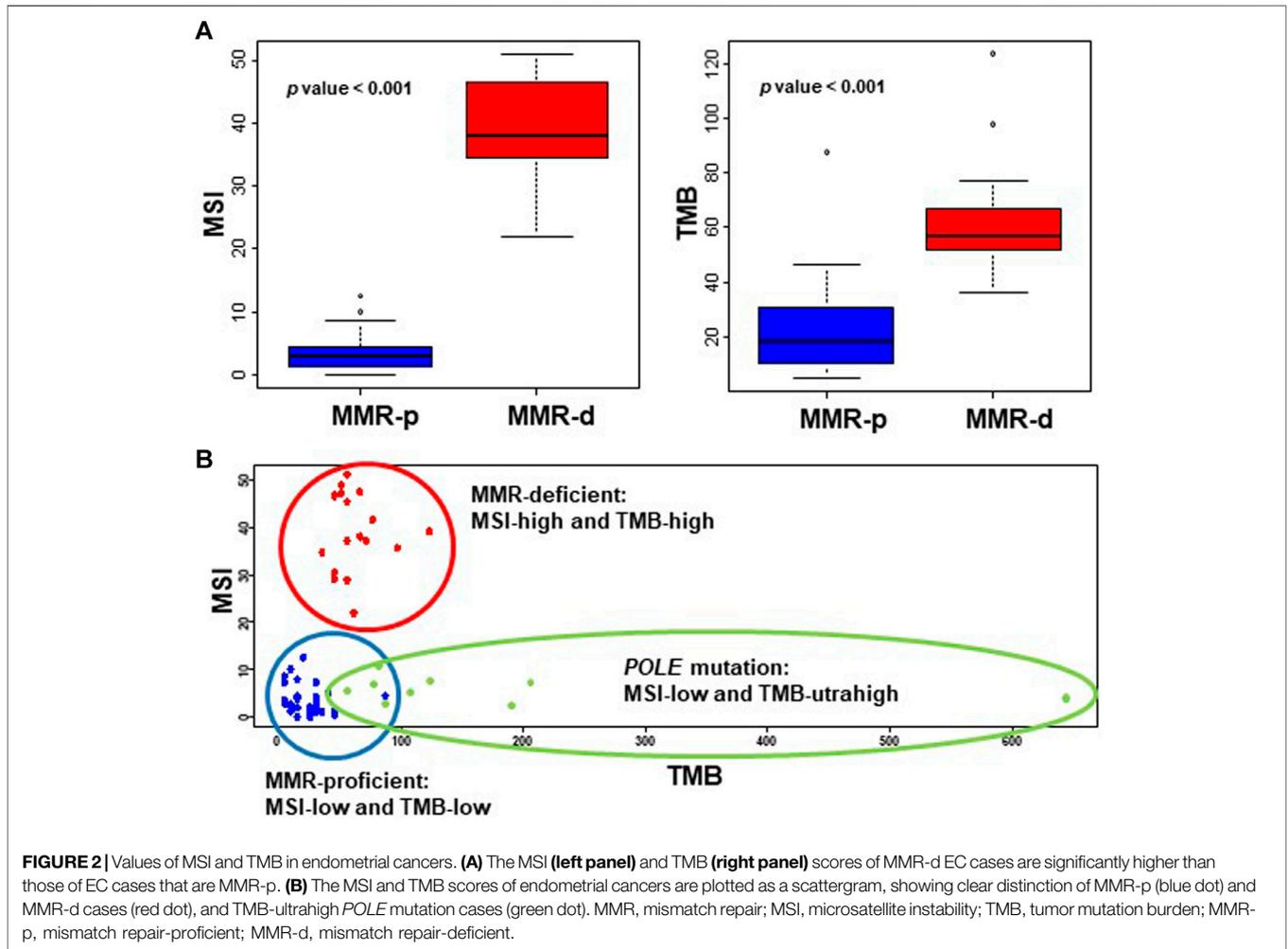


TABLE 2 | MSI and TMB in each histological subtype.

	n	MSI score	EC	CS	SC
DC	3	39.2 ± 5.5	$p = 0.037$	Ns	$p < 0.001$
EC	16	16.5 ± 5.0		Ns	ns
CS	4	20.5 ± 10.2			ns
SC	9	7.9 ± 3.6			

	n	TMB score	EC	CS	SC
DC	3	68.7 ± 4.6	ns	$p = 0.012$	$p = 0.013$
EC	16	43.2 ± 6.9		Ns	$p = 0.041$
CS	4	23.2 ± 11.6			ns
SC	9	21.8 ± 9.9			

The MSI, and TMB, scores are presented as mean ± standard error. EC with *POLE* mutation is excluded; MSI, microsatellite instability; TMB, tumor mutation burden; EC, endometrioid carcinoma; CS, carcinosarcoma; SC, serous carcinoma; ns, not significant.

reported [37-39], our study also demonstrated that CS and SC cases typically exhibited amplification in *MYC*, *CCND1*, and *CCNE1*. These findings were distinct from those in DC cases.

Using IHC as a tool for p53 and MMR protein analysis may facilitate differential diagnosis of DC and CS, as demonstrated by a recent study [24]. Our results substantiated this finding and

TABLE 3 | MSI and TMB in EC.

EC	n	MSI score	TMB score
MMR-p	10	2.3 ± 0.6	26.3 ± 3.5
MMR-d	6	40.4 ± 3.7	66.1 ± 12.8
p value		<0.0001	0.012

MSI, microsatellite instability; TMB, tumor mutation burden; EC, endometrioid carcinoma; MMR-p, mismatch repair-proficient; MMR-d, mismatch repair-deficient.

further elucidated the benefits associated with analysis of MMR proteins by IHC. However, some studies have indicated that CS exhibits a high rate of MMR-d cases (10–41%) [40, 41]. We propose that some DC cases, rather than true CS cases, may have been included in these studies, as many cases (>60%) demonstrated endometrioid morphology in the epithelial components [41]. Another study also demonstrated that DC cases were distributed in all TCGA classification categories [42]. The histological diagnosis of DC was based on the absence of E-cadherin expression and positive ZEB1 immunoreaction. These criteria were different from the diagnostic criteria used in our current study. In another report, a certain population of

TABLE 4 | Distribution of TCGA classification*.

	EC	DC	CS	SC	LCNEC
POLEmut	3	0	0	0	0
MMR-d	6	3	1	1	0
p53mut	2	0	2	8	1
NSMP	8	0	1	0	0
36	19	3	4	9	1

*Case no. 27, which exhibits both p53mut and MMR-d features, is categorized as p53mut type. EC, endometrioid carcinoma; DC, dedifferentiated endometrioid carcinoma; CS, carcinosarcoma; SC, serous carcinoma; LCNEC, neuroendocrine carcinoma; PLOEmut, POLE, mutation; MMR-d, mismatch repair-deficient; NSMP, no specific molecular profile; p53mut, p53 mutation.

endometrial DC exhibited concurrent loss of ARID1A and ARID1B and loss of SMARCA4 or SMARCB1 expression [43]. Therefore, the use of the histological criteria and immunophenotyping in DC remains controversial.

EC comprises glandular and solid (>5%) areas diagnosed as G2 or G3 EC, based on the percentage of the solid components and nuclear atypia [44]. In EC, solid components are more evenly distributed and typically have indistinct borders between glandular parts. Meanwhile, dedifferentiated solid areas in DC are well demarcated from glandular parts [1]. However, the differentiation of EC with solid components from DC is sometimes difficult, especially when diagnoses are based on histological findings alone. MMR-d was shared between wildtype POLE EC and DC. As G2/G3 EC commonly exhibits TP53 mutations secondary to MMR mutations [45], p53 IHC is not always useful for differentiating EC, DC, and CS from SC. Furthermore, like the SC case (no. 27) displayed both p53mut and MMR-d features, 3% of endometrial cancers exhibit other genomic features in addition to p53 abnormalities [45].

In accordance with previous studies [21, 22, 46], a high concordance was observed between ARID1A IHC and mutation status, while that between PTEN IHC and mutation was also high in EC but not in SC cases. Even IHC detects a greater population of PTEN loss than does NGS analysis [21], the frequency of SC cases showing loss of PTEN expression with no PTEN mutation might be unusually high in our study (five out of nine cases). The reported frequencies of SCs showing loss of PTEN expression range from 0 to 11% [47-50]. Since the method of PTEN IHC is often poorly reproducible [21, 51, 52], we cannot exclude a possibility of false negative PTEN IHC due to technical issues.

Compared with IHC-based analyses of MMR proteins, characteristic IHC profiles are not known for being used for the differential diagnosis of POLEmut EC from DC. The most characteristic feature of EC with POLEmut is ultrahigh TMB and low MSI scores [23]. However, to the best of our knowledge, a targeting antibody detecting mutated-POLE protein is unavailable, and no characteristic histological findings associated with POLEmut-type EC are presently known.

Overall, MLH1, POLD1, and TERT mutations in DC may be considered a characteristic molecular profile that can be used to distinguish it from EC. Pathogenic POLD1 mutations have been observed in colorectal cancers and polymerase proofreading-associated polyposis syndrome [53]. Compared with POLE, POLD1 mutations are less frequently observed in endometrial

cancers, although a few POLD1 mutations have been reported in G3 EC [54]. The sample size used to assess DC in this study was considerably small for determining the significance of TERT and POLD1 mutations for tumor cell dedifferentiation in EC.

As endometrial LCNEC also demonstrates undifferentiated solid elements, LCNEC should be considered in the differential diagnosis of endometrial cancer with solid proliferation. However, LCNEC diagnosis is not challenging due to its distinct IHC profile [55]. Herein, LCNEC was MMR-p and exhibited mt p53 and diffuse ARID1A expression, and its NGS profile was similar to that of SC. Although a previous study indicated that LCNEC should be classified as a variant of DC [56], a recent report has indicated that the nature of endometrial LCNEC is heterogeneous, and molecular analysis has shown that LCNEC may be grouped under any TCGA classification [57].

In summary, the feasibility of using a small NGS cancer panel to facilitate the molecular categorization of endometrial DC, SC, CS, and EC with solid proliferation was investigated. Genomic analyses that detect alterations in TMB, MSI, and gene mutations including MLH1, TERT, POLD1, and POLE, IHC analyses that assess p53 and MMR protein expression, and other conventional IHC including PTEN and ARID1A exhibit significant potential as a practical diagnostic tool for determining endometrial cancer pathology.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committees for Clinical and Epidemiologic Research at the Kagoshima University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YK and IK summarized the pathological and genomic data; TA analyzed and interpreted the sequencing data; KT contributed as a pathologist; SHY, MK, and ST summarized the clinical data; IS, SN, and SEY contributed to genomic data analysis; AT and HK organized the study and wrote the article; and all authors read and approved the final manuscript.

CONFLICT OF INTEREST

SN and IS are employed by Mitsubishi Space Software.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ACKNOWLEDGMENTS

The authors greatly appreciate the excellent technical assistance of Orié Iwaya and Mai Tokudome at the Department of Pathology, Kagoshima University Graduate School of Medical and Dental Sciences.

REFERENCES

- Kim K-R, Lax SF, Lazar AJ, Longacre TA, Malpica A, Matias-Guiu X, et al. Tumours of Uterine Corpus. In: *The WHO Classification of Tumours Editorial board Female Genital Tumours*. 5th ed. Lyon: IARC (2020). p. 245–308.
- Azumi N, Battifora H. The Distribution of Vimentin and Keratin in Epithelial and Nonepithelial Neoplasms: A Comprehensive Immunohistochemical Formalin- and Alcohol-Fixed Tumors. *Am J Clin Pathol* (1987) 88:286–96. doi:10.1093/ajcp/88.3.286
- Murali R, Davidson B, Fadare O, Carlson JA, Crum CP, Gilks CB, et al. High-grade Endometrial Carcinomas. *Int J Gynecol Pathol* (2019) 38(Suppl. 1): S40–S63. doi:10.1097/pgp.0000000000000491
- Piulats JM, Guerra E, Gil-Martín M, Roman-Canal B, Gatiús S, Sanz-Pamplona R, et al. Molecular Approaches for Classifying Endometrial Carcinoma. *Gynecol Oncol* (2017) 145:200–7. doi:10.1016/j.ygyno.2016.12.015
- Bell DW, Ellenson LH. Molecular Genetics of Endometrial Carcinoma. *Annu Rev Pathol Mech Dis* (2019) 14:339–67. doi:10.1146/annurev-pathol-020117-043609
- Carlson J, McCluggage WG. Reclassifying Endometrial Carcinomas with a Combined Morphological and Molecular Approach. *Curr Opin Oncol* (2019) 31:411–9. doi:10.1097/cco.0000000000000560
- Horak P, Fröhling S, Glimm H. Integrating Next-Generation Sequencing into Clinical Oncology: Strategies, Promises and Pitfalls. *ESMO Open* (2016) 1: e000094. doi:10.1136/esmoopen-2016-000094
- Chang Y-S, Huang H-D, Yeh K-T, Chang J-G. Identification of Novel Mutations in Endometrial Cancer Patients by Whole-Exome Sequencing. *Int J Mol Med* (2017) 50:1778–84. doi:10.3892/ijo.2017.3919
- Akahane T, Yamaguchi T, Kato Y, Yokoyama S, Hamada T, Nishida Y, et al. Comprehensive Validation of Liquid-Based Cytology Specimens for Next-Generation Sequencing in Cancer Genome Analysis. *PLoS One* (2019) 14: e0217724. doi:10.1371/journal.pone.0217724
- Yamaguchi T, Akahane T, Harada O, Kato Y, Aimono E, Takei H, et al. Next-generation Sequencing in Residual Liquid-based Cytology Specimens for Cancer Genome Analysis. *Diagn Cytopathology* (2020) 48:965–71. doi:10.1002/dc.24511
- Akahane T, Kitazono I, Yanazume S, Kamio M, Togami S, Sakamoto I, et al. Next-generation Sequencing Analysis of Endometrial Screening Liquid-Based Cytology Specimens: a Comparative Study to Tissue Specimens. *BMC Med Genomics* (2020) 13:101. doi:10.1186/s12920-020-00753-6
- Tafe LJ, Garg K, Chew I, Tornos C, Soslow RA. Endometrial and Ovarian Carcinomas with Undifferentiated Components: Clinically Aggressive and Frequently Underrecognized Neoplasms. *Mod Pathol* (2010) 23:781–9. doi:10.1038/modpathol.2010.41
- Vita G, Borgia L, Di Giovannantonio L, Bisceglia M. Dedifferentiated Endometrioid Adenocarcinoma of the Uterus. *Int J Surg Pathol* (2011) 19: 649–52. doi:10.1177/1066896911405987
- Raffone A, Travaglino A, Cerbone M, Gencarelli A, mollo A, Insabato L, et al. Diagnostic Accuracy of Immunohistochemistry for Mismatch Repair Proteins as Surrogate of Microsatellite Instability Molecular Testing in Endometrial Cancer. *Pathol Oncol Res* (2020) 26:1417–27. doi:10.1007/s12253-020-00811-5
- Xu C, Gu X, Padmanabhan R, Wu Z, Peng Q, DiCarlo J, et al. smCounter2: an Accurate Low-Frequency Variant Caller for Targeted Sequencing Data with Unique Molecular Identifiers. *Bioinformatics* (2019) 35:1299–309. doi:10.1093/bioinformatics/bty790
- Saotome K, Chiyoda T, Aimono E, Nakamura K, Tanishima S, Nohara S, et al. Clinical Implications of Next-generation Sequencing-based Panel Tests for

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.por-journal.com/articles/10.3389/pore.2021.1610013/full#supplementary-material>

- Malignant Ovarian Tumors. *Cancer Med* (2020) 9:7407–17. doi:10.1002/cam4.3383
- Galuppini F, Dal Pozzo CA, Deckert J, Loupakis F, Fassan M, Baffa R. Tumor Mutation burden: from Comprehensive Mutational Screening to the Clinic. *Cancer Cel Int* (2019) 19:209. doi:10.1186/s12935-019-0929-4
- Yarchoan M, Hopkins A, Jaffee EM. Tumor Mutational burden and Response Rate to PD-1 Inhibition. *N Engl J Med* (2017) 377:2500–1. doi:10.1056/nejmc1713444
- Niu B, Ye K, Zhang Q, Lu C, Xie M, McLellan MD, et al. MSIsensor: Microsatellite Instability Detection Using Paired Tumor-normal Sequence Data. *Bioinformatics* (2014) 30:1015–6. doi:10.1093/bioinformatics/btt755
- Johansen AFB, Kassetoft CG, Knudsen M, Laursen MB, Madsen AH, Iversen LH, et al. Validation of Computational Determination of Microsatellite Status Using Whole Exome Sequencing Data from Colorectal Cancer Patients. *BMC Cancer* (2019) 19:971. doi:10.1186/s12885-019-6227-7
- Djordjevic B, Hennessy BT, Li J, Barkoh BA, Luthra R, Mills GB, et al. Clinical Assessment of PTEN Loss in Endometrial Carcinoma: Immunohistochemistry Outperforms Gene Sequencing. *Mod Pathol* (2012) 25:699–708. doi:10.1038/modpathol.2011.208
- Khalique S, Naidoo K, Attygalle AD, Kriplani D, Daley F, Lowe A, et al. Optimised ARID1A Immunohistochemistry Is an Accurate Predictor of ARID1A mutational Status in Gynaecological Cancers. *J Pathol: Clin Res* (2018) 4:154–66. doi:10.1002/cjp.2.103
- Cancer Genome Atlas Research Network Kandoth C, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated Genomic Characterization of Endometrial Carcinoma. *Nature* (2013) 497:67–73. doi:10.1038/nature12113
- Jenkins TM, Hanley KZ, Schwartz LE, Cantrell LA, Stoler MH, Mills AM. Mismatch Repair Deficiency in Uterine Carcinosarcoma. *Am J Surg Pathol* (2020) 44:782–92. doi:10.1097/pas.0000000000001434
- Garg K, Shih K, Barakat R, Zhou Q, Iasonos A, Soslow RA. Endometrial Carcinomas in Women Aged 40 Years and Younger: Tumors Associated with Loss of DNA Mismatch Repair Proteins Comprise a Distinct Clinicopathologic Subset. *Am J Surg Pathol* (2009) 33:1869–77. doi:10.1097/pas.0b013e3181bc9866
- Han G, Sidhu D, Duggan MA, Arseneau J, Cesari M, Clement PB, et al. Reproducibility of Histological Cell Type in High-Grade Endometrial Carcinoma. *Mod Pathol* (2013) 26:1594–604. doi:10.1038/modpathol.2013.102
- McConechy MK, Ding J, Cheang MC, Wiegand KC, Senz J, Tone AA, et al. Use of Mutation Profiles to Refine the Classification of Endometrial Carcinomas. *J Pathol* (2012) 228:20–30. doi:10.1002/path.4056
- Cuevas D, Valls J, Gatiús S, Roman-Canal B, Estaran E, Dorca E, et al. Targeted Sequencing with a Customized Panel to Assess Histological Typing in Endometrial Carcinoma. *Virchows Arch* (2019) 474:585–98. doi:10.1007/s00428-018-02516-2
- Jones NL, Xiu J, Chatterjee-Paer S, Buckley de Meritens A, Burke WM, Tergas AI, et al. Distinct Molecular Landscapes between Endometrioid and Nonendometrioid Uterine Carcinomas. *Int J Cancer* (2017) 140:1396–404. doi:10.1002/ijc.30537
- Cherniack AD, Shen H, Walter V, Stewart C, Murray BA, Bowlby R, et al. Integrated Molecular Characterization of Uterine Carcinosarcoma. *Cancer Cell* (2017) 31:411–23. doi:10.1016/j.ccell.2017.02.010
- Chen X, Arend R, Hamele-Bena D, Tergas AI, Hawver M, Tong G-X, et al. Uterine Carcinosarcomas. *Int J Gynecol Pathol* (2017) 36:412–9. doi:10.1097/pgp.0000000000000346
- Talhok A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, et al. A Clinically Applicable Molecular-Based Classification for Endometrial Cancers. *Br J Cancer* (2015) 113:299–310. doi:10.1038/bjc.2015.190

33. Talhouk A, McConechy MK, Leung S, Yang W, Lum A, Senz J, et al. Confirmation of ProMisE: a Simple, Genomics-Based Clinical Classifier for Endometrial Cancer. *Cancer* (2017) 123:802–13. doi:10.1002/cncr.30496
34. Meyer LA, Broaddus RR, Lu KH. Endometrial Cancer and Lynch Syndrome: Clinical and Pathologic Considerations. *Cancer Control* (2009) 16:14–22. doi:10.1177/107327480901600103
35. Mills AM, Liou S, Ford JM, Berek JS, Pai RK, Longacre TA. Lynch Syndrome Screening Should Be Considered for All Patients with Newly Diagnosed Endometrial Cancer. *Am J Surg Pathol* (2014) 38:1501–9. doi:10.1097/pas.0000000000000321
36. Hoang LN, Lee Y-S, Karnezis AN, Tessier-Cloutier B, Almandani N, Coatham M, et al. Immunophenotypic Features of Dedifferentiated Endometrial Carcinoma - Insights from BRG1/INI1-Deficient Tumours. *Histopathology* (2016) 69:560–9. doi:10.1111/his.12989
37. Kuhn E, Wu R-C, Guan B, Wu G, Zhang J, Wang Y, et al. Identification of Molecular Pathway Aberrations in Uterine Serous Carcinoma by Genome-wide Analyses. *J Natl Cancer Inst* (2012) 104:1503–13. doi:10.1093/jnci/djs345
38. Leskela S, Pérez-Mies B, Rosa-Rosa JM, Cristobal E, Biscuola M, Palacios-Berraquero ML, et al. Molecular Basis of Tumor Heterogeneity in Endometrial Carcinosarcoma. *Cancers* (2019) 11:964. doi:10.3390/cancers11070964
39. Cuevas D, Velasco A, Vaquero M, Santacana M, Gatus S, Eritja N, et al. Intratumour Heterogeneity in Endometrial Serous Carcinoma Assessed by Targeted Sequencing and Multiplex Ligation-dependent Probe Amplification: a Descriptive Study. *Histopathology* (2020) 76:447–60. doi:10.1111/his.14001
40. Saijo M, Nakamura K, Ida N, Nasu A, Yoshino T, Masuyama H, et al. Histologic Appearance and Immunohistochemistry of DNA Mismatch Repair Protein and P53 in Endometrial Carcinosarcoma. *Am J Surg Pathol* (2019) 43:1493–500. doi:10.1097/pas.0000000000001353
41. De Jong RA, Nijman HW, Wijbrandi TF, Reyners AK, Boezen HM, Hollema H. Molecular Markers and Clinical Behavior of Uterine Carcinosarcomas: Focus on the Epithelial Tumor Component. *Mod Pathol* (2011) 24:1368–79. doi:10.1038/modpathol.2011.88
42. Rosa-Rosa JM, Leskelä S, Cristóbal-Lana E, Santón A, López-García MÁ, Muñoz G, et al. Molecular Genetic Heterogeneity in Undifferentiated Endometrial Carcinomas. *Mod Pathol* (2016) 29:1390–8. doi:10.1038/modpathol.2016.132
43. Coatham M, Li X, Karnezis AN, Hoang LN, Tessier-Cloutier B, Meng B, et al. Concurrent ARID1A and ARID1B Inactivation in Endometrial and Ovarian Dedifferentiated Carcinomas. *Mod Pathol* (2016) 29:1586–93. doi:10.1038/modpathol.2016.156
44. Zaino RJ, Kurman RJ, Diana KL, Paul Morrow C. The Utility of the Revised International Federation of Gynecology and Obstetrics Histologic Grading of Endometrial Adenocarcinoma Using a Defined Nuclear Grading System. A Gynecologic Oncology Group Study. *Cancer* (1995) 75:812–86. doi:10.1002/1097-0142(19950101)75:1<81:aid-cncr2820750114>3.0.co;2-f
45. León-Castillo A, Gilvazquez E, Nout R, Smit VT, McAlpine JN, McConechy M, et al. Clinicopathological and Molecular Characterisation of 'multiple-Classifer' Endometrial Carcinomas. *J Pathol* (2020) 250:312–22. doi:10.1002/path.5373
46. Wang L, Piskorz A, Bosse T, Jimenez-Linan M, Rous B, Gilks CB, et al. Immunohistochemistry and Next-Generation Sequencing Are Complementary Tests in Identifying PTEN Abnormality in Endometrial Carcinoma Biopsies. *Int J Gynecol Pathol* (2021). doi:10.1097/pgp.0000000000000763
47. Tashiro H, Blazes MS, Wu R, Cho KR, Bose S, Wang SI, et al. Mutations in PTEN Are Frequent in Endometrial Carcinoma but Rare in Other Common Gynecological Malignancies. *Cancer Res* (1997) 57:3935–40.
48. Risinger JI, Hayes K, Maxwell GL, Carney ME, Dodge RK, Barrett JC, et al. PTEN Mutation in Endometrial Cancers Is Associated with Favorable Clinical and Pathologic Characteristics. *Clin Cancer Res* (1998) 4:3005–10.
49. Sun H, Enomoto T, Fujita M, Wada H, Yoshino K, Ozaki K, et al. Mutational Analysis of the PTEN Gene in Endometrial Carcinoma and Hyperplasia. *Am J Clin Pathol* (2001) 115:32–8. doi:10.1309/7jx6-b9u9-3p0r-eqny
50. Rudd ML, Price JC, Fogoros S, Godwin AK, Sgroi DC, Merino MJ, et al. A Unique Spectrum of Somatic PIK3CA (P110α) Mutations within Primary Endometrial Carcinomas. *Clin Cancer Res* (2011) 17:1331–40. doi:10.1158/1078-0432.ccr-10-0540
51. Goebel EA, Vidal A, Matias-Guiu X, Blake Gilks C. The Evolution of Endometrial Carcinoma Classification through Application of Immunohistochemistry and Molecular Diagnostics: Past, Present and Future. *Virchows Arch* (2018) 472:885–96. doi:10.1007/s00428-017-2279-8
52. Maiques O, Santacana M, Valls J, Pallares J, Mirantes C, Gatus S, et al. Optimal Protocol for PTEN Immunostaining; Role of Analytical and Preanalytical Variables in PTEN Staining in normal and Neoplastic Endometrial, Breast, and Prostatic Tissues. *Hum Pathol* (2014) 45:522–32. doi:10.1016/j.humpath.2013.10.018
53. Church JM. Polymerase Proofreading-Associated Polyposis. *Dis Colon Rectum* (2014) 57:396–7. doi:10.1097/dcr.0000000000000084
54. Wong A, Kuick CH, Wong WL, Tham JM, Mansor S, Loh E, et al. Mutation Spectrum of POLE and POLD1 Mutations in South East Asian Women Presenting with Grade 3 Endometrioid Endometrial Carcinomas. *Gynecol Oncol* (2016) 141:113–20. doi:10.1016/j.ygyno.2015.12.031
55. Cree IA, Malpica A, McCluggage WG. Neuroendocrine Neoplasia. In: *The WHO Classification of Tumours Editorial board Female Genital Tumours*. 5th ed. Lyon: IARC (2020). p. 451–9.
56. Espinosa I, De Leo A, D'Angelo E, Rosa-Rosa JM, Corominas M, Gonzalez A, et al. Dedifferentiated Endometrial Carcinomas with Neuroendocrine Features: a Clinicopathologic, Immunohistochemical, and Molecular Genetic Study. *Hum Pathol* (2018) 72:100–6. doi:10.1016/j.humpath.2017.11.006
57. Howitt BE, Dong F, Vivero M, Shah V, Lindeman N, Schoolmeester JK, et al. Molecular Characterization of Neuroendocrine Carcinomas of the Endometrium. *Am J Surg Pathol* (2020) 44:1541–8. doi:10.1097/pas.0000000000001560

Copyright © 2021 Kobayashi, Kitazono, Akahane, Yanazume, Kamio, Togami, Nohara, Sakamoto, Yokoyama, Tabata, Kobayashi and Tanimoto. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.