

Published in final edited form as:

J Pathol. 2012 September ; 228(1): 20–30. doi:10.1002/path.4056.

Use of mutation profiles to refine the classification of endometrial carcinomas

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Abstract

The classification of endometrial carcinomas is based on pathological assessment of tumour cell type; the different cell types (endometrioid, serous, carcinosarcoma, mixed, and clear cell) are

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Authors have no conflict of interests to report.

Statement of author contributions

MKM, SPS, MH, BG and DGH conceived and designed the study. MKM, JD, JS, WY, MH, KT, TZ, and HM carried out experiments. BG and CHL performed pathological reviews. MM, JD, MCUC, KW, JS, WY, AT, LP, APS, DGM, JNM, SPS, PJG, BG, CHL and DGH collected data and performed data analysis and assisted in interpretation of data. MCUC, MKM, JD performed statistical analysis. MKM, JD, BG, CHL and DGH wrote the manuscript and created the figures. DGH and PJG provided endometrial samples for sequencing. All authors reviewed and approved the manuscript.

associated with distinct molecular alterations. This current classification system for high-grade subtypes, in particular the distinction between high-grade endometrioid (EEC-3) and serous carcinomas (ESC), is limited in its reproducibility and prognostic abilities. Therefore, a search for specific molecular classifiers to improve endometrial carcinoma subclassification is warranted. We performed target enrichment sequencing on 393 endometrial carcinomas from two large cohorts, sequencing exons from the following 9 genes; ARID1A, PPP2R1A, PTEN, PIK3CA, KRAS, CTNNB1, TP53, BRAF and *PPP2R5C*. Based on this gene panel each endometrial carcinoma subtype shows a distinct mutation profile. EEC-3s have significantly different frequencies of *PTEN* and *TP53* mutations when compared to low-grade endometrioid carcinomas. ESCs and EEC-3s are distinct subtypes with significantly different frequencies of mutations in *PTEN*, *ARID1A*, *PPP2R1A*, *TP53*, and *CTNNB1*. From the mutation profiles we were able to identify subtype outliers, i.e. cases diagnosed morphologically as one subtype but with a mutation profile suggestive of a different subtype. Careful review of these diagnostically challenging cases suggested that the original morphological classification was incorrect in most instances. The molecular profile of carcinosarcomas suggests two distinct mutation profiles for these tumours; endometrioid-type (*PTEN*, *PIK3CA*, *ARID1A*, *KRAS* mutations), and serous-type (*TP53* and *PPP2R1A* mutations). While this nine gene panel does not allow for a purely molecularly based classification of endometrial carcinoma, it may prove useful as an adjunct to morphological classification and serve as an aid in the classification of problematic cases. If used in practice, it may lead to improved diagnostic reproducibility and may also serve to stratify patients for targeted therapeutics.

Keywords

Endometrial carcinoma; uterine; mutation profiles; endometrioid; serous; carcinosarcoma; classification

Introduction

The incidence of endometrial carcinoma is rising in the western world, and it is currently the most common type of gynaecological carcinoma [1]. This increase has been linked to increased obesity, increased life expectancy and tamoxifen use in women [2]. The classical pathogenic dualistic model proposed by Bokhman in 1983, placed endometrial carcinomas into one of two groups; estrogen-dependent endometrioid carcinomas, and estrogen-independent non-endometrioid carcinomas [3]. The classification of endometrial carcinomas used in clinical practice is based on histopathological assessment to determine cell type and grade [4-5], and is used in guiding therapy [6-7]. Endometrioid endometrial carcinomas (EECs) represent 70-80% of cases, are generally low grade (grade 1 or 2) with favourable prognosis, and most are cured by hysterectomy alone [8-9]. However, less common high-grade (grade 3) endometrioid carcinomas (EEC-3) have a significantly worse prognosis [5, 10]. The remaining 20-30% of non-endometrioid subtypes consist mostly of serous, and less commonly carcinosarcoma (previously known as MMMT or mixed malignant mullerian tumours), mixed histology, and clear cell carcinomas. These non-endometrioid tumours are not generally graded in the WHO grading system [6], are considered high-grade, as they are associated with poor outcomes [11]. Recent reports have shown the current pathological classification and grading system of high-grade endometrial carcinomas is limited in both reproducibility and prognostic ability [10, 12-14].

Molecular alterations in the PI3K/AKT, MAPK, and WNT signalling pathways have been implicated in the pathogenesis of specific endometrial carcinoma subtypes [15-18]. Thus there is a rationale for using mutational profiles in the classification of these tumours. EECs are molecularly recognized by frequent mutations in *PTEN*, *PIK3CA*, *KRAS*, *CTNNB1*,

FGFR2, and microsatellite instability (MSI) [8, 19-22]. Recent studies have identified mutations in *ARID1A* [24], *PIK3R1* [23], and *PIK3R2* [24] in EECs. Endometrial serous carcinomas (ESCs), and carcinosarcomas characteristically do not harbour a high frequency of these mutations, however, *TP53* [8, 19-20], and *PPP2R1A* [25-26] mutations are known to be common in ESC. *TP53* mutations are also detected in carcinosarcomas [27] and EEC-3s [10, 28].

Next-generation sequencing technologies has allowed sequencing of multiple genes and samples simultaneously [24], making large mutational studies achievable. As no single gene is a sensitive or specific marker for endometrial carcinoma subtypes, it is likely that the analysis of gene panels will be needed to guide subclassification. The aim of this study was to determine the mutation profiles of a large series of endometrial carcinomas, based on oncogenes and tumour suppressor genes known to be important in carcinogenesis, in an attempt to improve the classification of endometrial carcinomas.

Materials and Methods

Patient Samples

We obtained 152 endometrial tumours, and 90 corresponding buffy coat specimens originating from the BC Cancer Agency and Vancouver General Hospital via the OvCaRe Tissue Biobank repository, Vancouver, BC, Canada. Patients were informed for written consent, and research ethics approved as previously described [25]. An additional 260 endometrial tumour DNA samples were obtained from Washington University, St. Louis, Missouri. The endometrial subtype, grade and microsatellite instability data was previously determined in these cases. All samples from both centers' have undergone review by gynaecological pathologists.

Exon Sequencing

Genomic DNA (500ng) was used for indexed Illumina library construction [29], then underwent targeted enrichment using biotinylated RNA capture probes generated from cDNA clones or PCR amplicons [30] representing exons of *ARID1A*, *PTEN*, *PIK3CA*, *KRAS*, *CTNNB1*, *PPP2R1A*, *BRAF*, *TP53*, and *PPP2R5C* and sequenced using Illumina (GAIIx).

Bioinformatics Analysis

Short reads were aligned to the human genome (hg18) using the BWA aligner v0.5.9 [31]. A Random Forest classifier trained on validated SNVs was used to remove false-positive calls [32]. SNVs in the Catalogue of Somatic Mutations in Cancer (COSMIC) [33] were considered to be true positives, so a 99% cutoff threshold was selected (Figure S1). Mean coverage was plotted for cases with and without mutations (Figure S2). Details found in Supplementary materials and methods.

DNA validations

Select predicted SNVs were validated using Sanger sequencing as previously described [25]. See Supplementary materials and methods.

Identifying outlier cases

Outliers were identified by observing mutation profiles that did not fit the original diagnosed histological subtype; defined as ESC with *PTEN* and/or *ARID1A* mutations, and low-grade EECs with only *TP53* and/or *PPP2R1A* mutations. With the goal of comparing mutational outliers with immuno-profiles, formalin-fixed embedded paraffin blocks were only available

for 147/156 Vancouver cases, for the construction of a Tissue Microarray (TMA). For details see Supplementary materials and methods. These cases were used for the characterization of mutational outliers, by correlating with morphology and immunohistochemistry (IHC), and retrospectively reviewed by two independent pathologists, using the full hysterectomy case, without knowledge of mutation or IHC data.

Statistical Analysis

Fisher exact tests and multivariable logistic regression analysis were used to test the significance of associations between mutations within subtypes. All tests were two-tailed and p-value < 0.05 were considered significant. Fisher exact tests were not adjusted for multiple comparisons. The multivariable logistic regression model used step-wise selection based on the likelihood ratio test, with all genes included. The Hosmer-Lemeshow test was used to assess the goodness-of-fit of the estimated logistic regression models.

Results

To determine the mutation frequencies in various subtypes of endometrial carcinomas, we used exon capture sequencing of *ARID1A*, *PTEN*, *PIK3CA*, *KRAS*, *CTNNB1*, *PPP2R1A*, *BRAF*, *TP53*, and *PPP2R5C*. This resulted in the detection of somatic nonsynonymous missense, truncating, indels (insertions/deletions), and splice site mutations in 90.1% (353/392) of cases. The characteristics of the endometrial carcinomas, with histology subtypes and grade, are summarized in Table 1. We have stratified these carcinomas into low-grade (grade 1 and 2) EECs, EEC-3, ESC, carcinosarcoma, mixed, and undifferentiated, based on routine histopathological assessment, to determine the differences in mutational profiles. All mutational data are summarized in Table S1. The mutation frequencies of *ARID1A*, *PTEN*, *PIK3CA*, *PPP2R1A*, *TP53*, and *CTNNB1* are significantly different across four subtypes of endometrial carcinomas (Table 2).

High-grade and low-grade endometrioid carcinomas have similar mutation profiles but differ in frequencies of *TP53* mutations

Low-grade EECs have high to moderate frequencies of mutations in *PTEN*, *ARID1A*, *PIK3CA*, and *CTNNB1* (Table 2), with a higher frequency of mutations of *PTEN*, *ARID1A*, *PIK3CA*, *KRAS*, *PPP2R1A* and *TP53* seen in EEC-3s (Table 2). The comparison of mutations in low-grade EEC and EEC-3 showed that *PTEN* (p=0.0111) and *TP53* (p=0.0046) mutation frequencies are significantly different (Table 3). Multivariable logistic regression also revealed that *PTEN* (p=0.007) and *TP53* (p<0.001) mutations significantly distinguish EEC-3 from low-grade EEC (Table 4).

Endometrial serous carcinomas show a distinct mutation profile

Of 37 ESCs, high frequencies of mutations were found in *TP53*, *PPP2R1A*, and *PIK3CA* (Table 2). *TP53* and/or *PPP2R1A* mutations were found in 28/37 (75.7%) of ESCs, accounting for the majority of aberrations in this subtype (Figure 1). The comparison of EEC-3 to ESC revealed significantly different mutation frequencies for *ARID1A*, *PTEN*, *PIK3CA*, *CTNNB1*, *PPP2R1A*, and *TP53* (p <0.05) (Table 3). Low frequencies to zero mutation events were noted for some genes common in both ESCs and EEC-3. In an attempt to keep all the multivariate analyses consistent across the subtype comparisons, we included the same list of genes in the logistic regression model building between EEC-3 and ESC. As a result, there was no one reliable multivariable logistic regression model built, based on the mutation markers, to distinguish between these two subtypes (Table 4). As expected, the mutational profiles of low-grade EEC and ESC are significantly different (Table 3). Multivariable logistic regression shows, *PTEN* (p <0.001) with a trend of *ARID1A* (p=0.08)

mutations associated with low-grade EEC, whereas *PPP2R1A* and *TP53* ($p < 0.001$) are associated with ESC (Table 4).

Cases with discordant morphological diagnosis and mutational profiles

As discussed, ESCs were found to have a high frequency of mutations in *TP53* and *PPP2R1A* (Figure 1). From the mutation profiles we identified three histology-defined ESC cases with *ARID1A* and *PTEN* mutations and lacked *TP53* mutations, a profile more indicative of EECs (Figure 1). Other studies have not found *ARID1A* or *PTEN* mutations in ESCs, however there have been limited studies testing for *ARID1A* mutations in endometrial carcinomas [34-36]. On independent histopathological review of these three cases, all were mixed tumours consisting predominantly of ESC, but with minor components of low-grade EEC in two cases, and EEC-3 with clear cell carcinoma in one case (Table 5). For the two mixed ESC and low-grade EEC cases, we confirmed the section of tumour sample used for DNA extraction and subsequent sequencing exclusively contained the ESC component (Figure 2); however it harboured mutations with an endometrioid profile. Immunostaining is recommended for use in diagnostically problematic cases [37], although not universally used. These three cases showed a non-serous IHC profile; p53 normal expression and p16 negative expression, while one expressed ER and PR (Table 5).

We also identified four outlier low-grade EECs that contained *TP53* mutations and lacked *PTEN* mutations, which were also diagnostically challenging cases. Upon review, two cases showed morphological features of serous, and one case was re-classified from low-grade EEC to EEC-3. One outlier remained classified as low-grade EEC, however it was noted that this case showed extensive myometrial invasion and widespread lymphovascular invasion. By IHC, abnormal p53 expression was confirmed in all cases. All were, however, ER-positive with *PTEN* loss of expression, features found primarily in EECs. In two of these cases, p16 was strongly expressed (Table 5). In summary, these seven outlier cases showed features intermediate between ESC and EEC in morphological, IHC and genetic analysis (Table 5, Table S2).

We also performed unsupervised hierarchical clustering analysis on the 147 cases with IHC and mutational status (Figure S3, Table S2). This shows most low-grade EEC and EEC-3 subtypes cluster together, while the remaining EEC-3, serous and mixed cases are scattered. The mutational outliers with the diagnosed subtype are indicated, as well as the new classification.

Carcinosarcomas show either an endometrioid or serous mutation profile

Endometrial carcinosarcomas are relatively rare, and their classification as an endometrial carcinoma subtype or as a distinct entity is under debate [38]. In our analysis, of carcinosarcomas we found mutations in *TP53*, *PTEN*, *PIK3CA*, *ARID1A*, and *PPP2R1A* (Table 2). Two subgroups of carcinosarcomas were identified; one group characterized by mutations in *PTEN* and *ARID1A* (endometrioid-type), and a second group with *TP53* and *PPP2R1A* mutations more similar to ESC (Figure 1). Heterologous differentiation of the sarcomatous component was observed in a subset of tumors from both groups. Histopathological reviews of cases were not available; therefore it was not possible to correlate morphological features and mutational profiles of endometrioid-like or serous-like in the epithelial components of these tumours.

Mutations involving signalling pathways in endometrial carcinomas

By mutational analysis of multiple genes, it is possible to identify different mutations involving a single signalling pathway that may be functionally equivalent, and to examine the relationship between mutations involving different genes/pathways. Mutations in the

PI3K and MAPK signalling pathways are known to be important in EECs, therefore we further examined the prevalence of mutations in *PTEN*, *PIK3CA*, *KRAS*, *ARID1A* and *CTNNB1*. We found 211/276 (76.5%) low-grade EECs have *PTEN* and/or *PIK3CA* mutations (Figure 1). Co-existent *PTEN* and *PIK3CA* mutations were identified in 79/276 (28.6%) low-grade EECs, and 16/30 (53.3%) EEC-3s ($p=0.0112$). *ARID1A* mutations have recently been identified in low-grade EECs; however the relationship of these mutations with other pathways such as PI3K and WNT has not been examined [34]. Of the low-grade EECs with *ARID1A* mutations, 112/129 (86.8%) have mutations within *PTEN* and/or *PIK3CA* ($p=0.0002$). EEC-3s with *ARID1A* mutations ($n=18$) all have *PTEN* mutations, and 13/18 (72.2%) also have *PIK3CA* mutations. Thus there is a significant association between *ARID1A* and *PTEN/PIK3CA* mutations.

Microsatellite instability

MSI is a feature of the endometrioid subtype, therefore we determined the MSI status of 241/276 low-grade EECs and 13/30 EEC-3s. We found 97/241 (40.2%) of the low-grade EECs are MSI positive, compared to 8/13 (61.5%) of EEC-3 (Table S1).

Discussion

Endometrial carcinoma is a heterogeneous disease, comprised of multiple subtypes with differing risk factors, precursor lesions, and outcomes. Lack of reproducibility in histopathological diagnosis of endometrial carcinoma subtypes has hindered progress. For example, while some studies have found that EEC-3 and ESC have different outcomes [39], other studies have not [10]. This difference may reflect inclusion of different cases, based on subtly different diagnostic criteria, within these cohorts. Robust and reproducible diagnostic categories are an important first step in moving towards subtype-specific treatment, as is happening for ovarian carcinoma [40-41]. However in the case of endometrial carcinoma, it is likely that molecular markers will be needed to improve the suboptimal performance of conventional histopathological assessment [42]. With the advent of next-generation sequencing technologies, the molecular profiles of many tumour cell types are being extensively characterized. The knowledge of these mutation profiles can potentially be used diagnostically for subclassification, and to identify relevant targets for the development/deployment of targeted therapeutics. In this study, we performed exon capture sequencing of nine genes in two large cohorts of endometrial carcinomas, revealing differing mutational landscapes for endometrial carcinoma subtypes.

As demonstrated in previous studies, we identified high frequencies of mutations within *PTEN*, *PIK3CA*, *ARID1A*, *KRAS* and *CTNNB1*, and lack of *TP53* mutations in low-grade EECs. EEC-3s demonstrate a similar pattern of mutations, but with a significantly increased frequency of *TP53* mutations. High frequencies of *PTEN* mutations in EECs confirm this is an early driver event in tumour progression. Our results show that the frequency of MSI cases is similar in low-grade EEC and EEC-3, which supports the view that the majority of EEC-3s have progressed from low-grade EEC [10].

Recent studies identified a high frequency of concurrent *PTEN* and *PIK3CA* mutations in endometrial carcinomas [15, 24], but not in any other tumour type investigated to date [24]. In this study, we also observed this phenomenon in low-grade EECs and EEC-3s, but not in ESC or carcinosarcoma. We have determined that in low-grade EECs and EEC-3s, *ARID1A* mutations are significantly associated with concurrent mutations in *PTEN* and *PIK3CA*, a novel finding suggesting a cooperative role of these pathways in EEC tumourigenesis.

ESCs have frequent *TP53* and *PPP2R1A* mutations, and lack mutations in *PTEN*, *ARID1A* and *CTNNB1*, a mutational profile distinct from that of EECs. While it was not possible to

classify tumours solely based on this nine gene mutation panel, we were able to use the mutation profile as a diagnostic adjunct for morphological subclassification in individual cases. This is an attractive prospect given the significant problems in distinguishing EEC-3 and ESC highlighted in recent studies [5, 13-14, 28, 37, 43]. We observed mutational outliers where the original diagnosis did not fit the mutation profile, specifically ESC cases with *ARID1A* mutations, and low-grade EECs with only *TP53* mutations. In most of these outlier cases, retrospective review by two independent pathologists resulted in reclassification, agreeing with the subtype-specific mutation patterns rather than the original diagnosis.

It has previously been proposed that ESC may arise through two different tumourigenic pathways, i.e. from progression through hyperplasia and low-grade EEC, or arising via high-grade endometrial intraepithelial carcinoma, in an estrogen-independent pathway [44]. In this study, we observed two tumours initially diagnosed as ESC that showed an endometrioid mutation profile. On retrospective review the diagnosis for both was changed to mixed serous and endometrioid. This observation is not novel but does give further support to ESCs arising in some cases by an alternative molecular pathway, rather than the classical Type 2 pathway (Figure 3, Figure S4) [9]. This further suggests that the classification of endometrial carcinomas cannot be encompassed by a simple dualistic model. In particular, the high-grade subtypes show considerable heterogeneity not reflected adequately in a Type 1 versus Type 2 model. Future studies will be required to address the following issues: 1. How reproducible is molecularly supported subtype diagnoses? 2. If diagnoses can be made reproducibly, do subtypes show significant differences in stage at diagnosis, pattern of spread, prognosis or response to treatment? Only after those questions are addressed can subtype-specific management move forward, and mutation-based treatment decisions can be made for challenging diagnoses.

We also investigated the molecular profiles of carcinosarcomas. These tumours are generally rare with poor prognosis [45], and are composed of a mixture of carcinomatous and sarcomatous elements [46]. While previous studies have not identified a high number of mutations in this subtype [47], we have shown a moderate frequency of mutations in the majority of genes sequenced. This discrepancy may be due to limited exon sequencing in previous studies; in the current study all exons of these genes were interrogated. Two patterns of mutations were observed; an endometrioid-type mutation profile (*ARID1A*, *PTEN*, *PIK3CA*, *KRAS*) or a serous mutation profile (*TP53*, *PPP2RIA*). This suggests a dualistic molecular evolution of carcinosarcomas with an endometrioid-like or serous-like mutation pattern (Figure 3). Further validation studies will be necessary to determine if these molecular profiles are associated with different morphological features in the carcinomatous or sarcomatous components, or are associated with outcome differences.

We acknowledge that there are limitations of this study; we were unable to perform full histopathological reviews of many cases, including all carcinosarcomas. There were also limited numbers of cases of EEC-3 and ESC in this study, therefore independent validation studies, linked with outcome [48], will be needed in these tumour types. There is also uncertainty about the sensitivity of the exon capture method, and false negatives are likely to be present in this data set. The TCGA endometrial sequencing effort will prove to be useful in validating the observations of this study.

In conclusion, we have identified distinct molecular profiles that may aid in endometrial carcinoma classification leading to more reproducible diagnoses. Although endometrial carcinoma subtypes diagnoses and grade are currently used in guiding patient management, mutational analysis is emerging as a realistic option in clinical practice. In the future, we predict that the mutational classification of endometrial carcinomas will become an

important tool in diagnosis, guiding mutation-based targeted treatment decisions. Mutation profiles are already being applied in other cancers for selecting targeted therapeutics, for example BRAF inhibitors in malignant melanoma [49] and BRAF and EGFR targeting in colorectal cancers [50-51]. Determination of the role of mutational analysis in assessment of endometrial carcinomas will require additional study, with careful comparison of molecular versus conventional subclassification.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work is supported by grants from the British Columbia (BC) Cancer Foundation and the Vancouver General Hospital (VGH)–University of British Columbia Hospital Foundation (to the OvCaRe ovarian cancer research team in Vancouver) and the Canadian Institutes of Health Research (CIHR). Work supported at Washington University in part by RO1CA71754 and P50CA134254 to PJG. We thank all the women who donated the samples used in this study. In addition, we would like to thank Jason Madore, Christine Chow and Sally Cheng and the Sequence Production and LIMS groups at Canada's Michael Smith Genome Sciences Centre for technical assistance; and the fellows of the University of British Columbia Gynaecologic Oncology Program for obtaining consent from patients for data in the OvCaRe tumour bank.

List of Abbreviations

EEC	Endometrial Endometrioid Carcinoma
EEC-3	High-grade (grade 3) Endometrial Endometrioid Carcinoma
ESC	Endometrial Serous Carcinoma
TMA	Tissue Microarray
MSI	Microsatellite Instability
TCGA	The Cancer Genome Atlas

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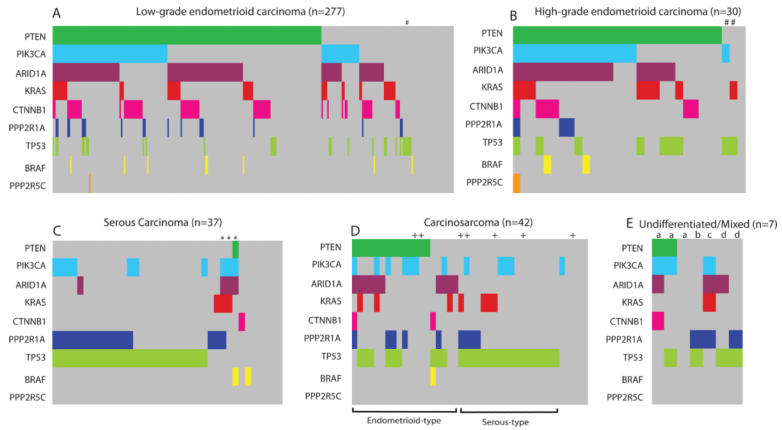


Figure 1. Mutation profiles of endometrial subtypes

A. Low-grade endometrioid carcinoma, including grade 1 and 2 tumours (n=276); **B.** High-grade endometrioid carcinoma, grade 3 tumours (n=30); **C.** Serous carcinoma (n=37); **D.** Carcinosarcoma (n=42), (+) indicates carcinosarcomas with heterologous differentiation elements; **E.** Undifferentiated and mixed histology subtypes, (a) undifferentiated carcinomas, (b) mixed low-grade EEC with serous carcinoma, (c) mixed endometrioid and clear cell carcinoma, (d) mixed serous and clear cell carcinoma. Rows indicate genes, columns represent tumour cases. Coloured bars indicate mutations' including; missense, truncating, indels and splice site mutations. Grey bars indicate no mutations were detected. (*) indicates serous carcinoma outliers with *ARID1A* mutations; (#) indicates low-grade EECs and EEC-3s mutation outliers with serous-type mutations (*TP53* or *PPP2R1A*).

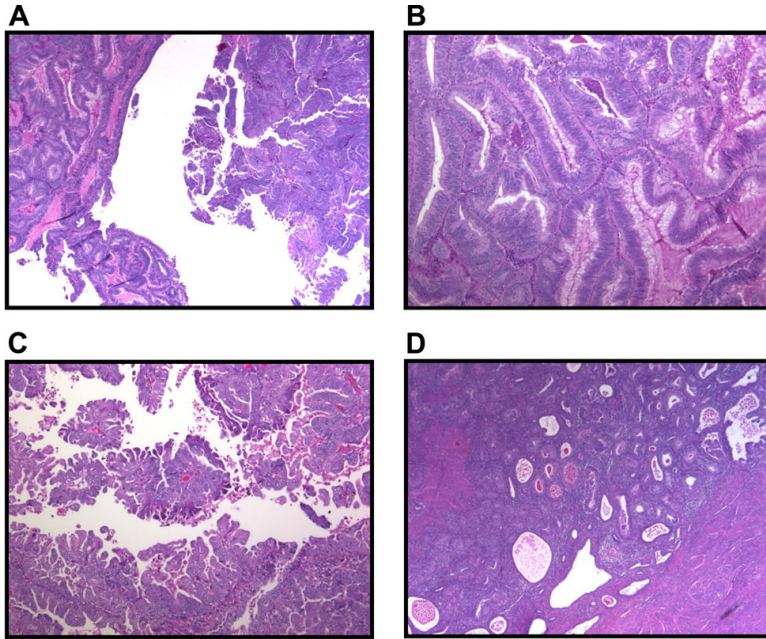


Figure 2. A case originally diagnosed as serous carcinoma, but with an *ARID1A* mutation and no *TP53* mutation, is a mixed low-grade endometrioid and serous carcinoma (case #1120)
A. A mix of a grade 1 endometrioid (left half) and high-grade serous (right half) carcinoma, 40X magnification; B. High power (100X) image of histologically distinct low-grade endometrioid carcinoma; C. High power (100X) images of serous carcinoma component, of which the sampling of tumour was used for mutation sequencing; D. Atypical complex hyperplasia in the background endometrium 40X magnification.

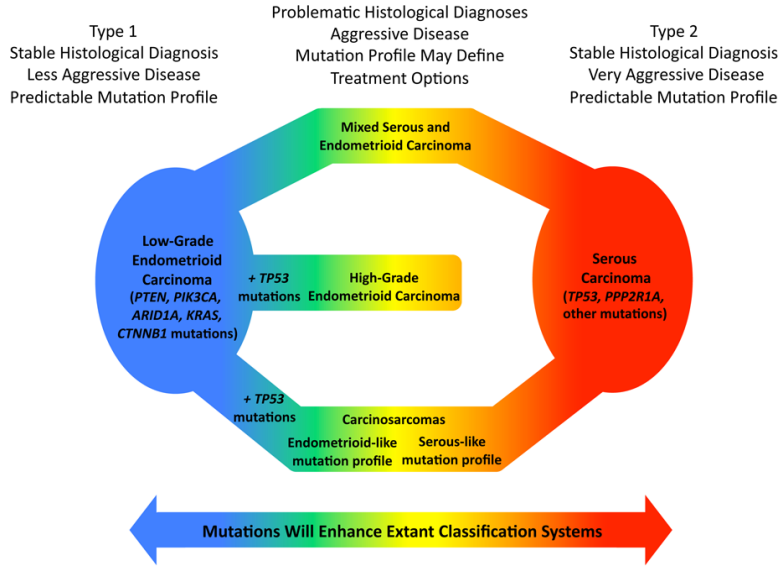


Figure 3. Mutational analysis may be an effective tool to classify morphologically problematic cases into biologically relevant treatment groups
 Intermediate high-grade cell types tend to be diagnostically challenging cases, often with multiple morphological features of endometrioid and/or serous carcinomas. The addition of mutation profiles can lead to reproducible diagnosis and the future of mutation-based treatment decisions for targeted therapeutics. Blue and red colours indicate distinct mutation profiles for low-grade EEC and serous carcinomas. Yellow indicates the cases where the mutational profiles will aid in separating out the appropriate histological subtype and dictate appropriate treatment options for patients.

Table 1

Summary of all endometrial carcinoma subtypes.

All Subtypes	
Endometrioid	306
Grade 1	169
Grade 2	107
Grade 3	30
Serous	37
Mixed *	4
Undifferentiated	3
Carcinosarcoma	42
Total	392

* Includes one cases as mixed serous and endometrioid carcinoma, one case mixed G2 and G3 endometrioid and clear cell carcinoma, and two cases as mixed serous and clear cell carcinoma.

Table 2

The frequency of mutations within all endometrial subtypes.

	Low-Grade Endometrioid (G1 and 2) (n=276)	High-Grade Endometrioid (G3) (n=30)	Serous (n=37)	Carcinosarcoma (n=42)	p-value across all subtypes (chi- squared test)
PTEN	185 (67.0%)	27 (90.0%)	1 (2.7%)	14 (33.3%)	4.63E-17
PIK3CA	105 (38.0%)	17 (56.7%)	10 (27.0%)	12 (28.6%)	0.0480
ARID1A	129 (46.7%)	18 (60.0%)	4 (10.8%)	10 (23.8%)	5.77E-06
KRAS	46 (16.6%)	8 (26.7%)	3 (8.1%)	7 (16.7%)	0.2434
CTNNB1	66 (23.8%)	6 (20.0%)	1 (2.7%)	2 (4.8%)	1.19E-03
PPP2R1A	19 (6.9%)	3 (10.0%)	16 (43.2%)	9 (21.4%)	1.50E-09
TP53	28 (10.1%)	9 (30.0%)	25 (67.6%)	27 (64.3%)	2.79E-23
BRAF	8 (2.9%)	2 (6.7%)	2 (5.4%)	1 (2.4%)	0.6186
PPP2R5C	1 (0.4%)	2 (6.7%)	0 (0%)	0 (0%)	0.002

Bold indicates significant p-values <0.05

Table 3

Univariate Fisher exact test (p-values) to show significant differences between mutation profiles of each endometrial carcinoma subtypes.

	Low-Grade Endometrioid vs High-Grade Endometrioid	Low-Grade Endometrioid vs Serous	High-Grade Endometrioid vs Serous	High-Grade Endometrioid vs Carcinosarcoma	Serous vs Carcinosarcoma
PTEN	0.0111	6.58E-15	2.57E-14	1.09E-06	4.30E-04
PIK3CA	0.0522	0.2091	0.0235	0.0276	1.0000
ARID1A	0.1826	1.38E-05	2.42E-05	0.0030	0.1522
KRAS	0.2000	0.2328	0.0525	0.3814	0.3215
CTNNB1	0.8211	1.23E-03	0.0394	0.0602	1.0000
PPP2R1A	0.4630	4.96E-08	2.95E-03	0.3365	0.0526
TP53	4.62E-03	8.56E-14	3.17E-03	0.0080	0.8151
BRAF	0.2555	0.3352	1.0000	0.5669	0.5972
PPP2R5C	0.0263	1.0000	0.1967	0.1702	NA

Bold indicates significant p-values <0.05

Table 4

Multivariable logistic regression analysis of gene mutations between endometrial carcinoma subtypes. Reported values are only the most significant genes selected by the step-wise selection method based on the Likelihood ratio test.

Gene (Marker)	Low-grade Endometrioid (n=276) vs. High-grade Endometrioid (n=30)	Low-grade Endometrioid (n=276) vs. Serous (n=37)	High-grade Endometrioid (n=30) vs. Serous (n=37)	High-grade Endometrioid (n=30) vs. Carcinosarcoma (n=42)	Serous (n=37) vs. Carcinosarcoma (n=42)			
	p-value	Odds ratio* to high-grade endometrioid	p-value	Odds ratio* to serous	p-value	Odds ratio* to carcinosarcoma	p-value	Odds ratio* to carcinosarcoma
PTEN	0.007	5.61 (1.6-19.7)	1.89E-04	0.02 (0.002-0.14)			6.24E-03	19.41 (2.3-162.6)
PIK3CA								
ARID1A			0.080	0.3 (0.08-1.2)				
KRAS								
CTNNB1								
PPP2R1A				13.28 (3.3-53.4)				
TP53	7.04E-04	4.95 (2.0-12.5)	2.7E-04	7.64 (2.8-20.9)				
BRAF			1.40E-02	18.9 (1.8-196.7)			0.0736	5.12 (0.86-30.7)
PPP2R5C								0.0446

* Odds ratio (95% CI)

Table 5

Outlier cases with pathological review, IHC and mutation profile.

ID	841	1120	220	895	511	1034	611
Original Subtype	Serous carcinoma	Serous carcinoma	Serous carcinoma	Low-grade endometrioid carcinoma	Low-grade endometrioid carcinoma	Low-grade endometrioid carcinoma	Low-grade endometrioid carcinoma
Review	mixed serous (80%) and low-grade endometrioid carcinoma, with adjacent endometrium showing focal complex atypical hyperplasia	mixed serous (60%) and low-grade endometrioid carcinoma, with adjacent endometrium showing complex atypical hyperplasia	Grade 3 endometrioid with clear cell changes	Grade 2 endometrioid (extensively myometrial-invasive and LVI)	Grade 3 endometrioid	Mixed low-grade (G2) endometrioid and serous carcinoma	Serous carcinoma
p53-IHC ^a	1	1	1	2	2	2	2
ER-IHC ^b	0	1	0	1	1	1	1
P16-IHC ^b	0	0	0	0	0	1	1
PR-IHC ^b	0	1	0	1	1	1	NA
PTEN-IHC ^c	1	1	0	0	0	0	0
ARID1A	p.Q420*, p.R1335*	p.Q2176fs	p.Q548fs, p.G1847fs				
PTEN			p.L265fs			splice site acceptor	
PIK3CA	p.G106V, p.V344M	p.Q546K, p.H1047Y	Y1021C				
KRAS	p.G13D	p.G12A					
PPP2R1A	p.R182W						
TP53				p.R282W	p.P179L	p.R248Q	p.S241F
CTNNB1					p.H193L		
BRAF			p.A526V, p.P403fs				
PPP2R5C							

^aScoring: 0= loss of expression, 1= normal expression, 2= over-expression

^bScoring: 0= no expression, 1= over-expression

^cScoring: 1=normal expression, 0= loss of expression