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TP53 Mutational Spectrum in Endometrioid and Serous Endometrial Cancers

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SUMMARY

Endometrial carcinomas (ECs) are heterogeneous at the genetic level. Whilst *TP53* mutations are highly recurrent in serous endometrial carcinomas (SECs), these are also present in a subset of endometrioid endometrial carcinomas (EECs). Here we sought to define the frequency, pattern, distribution and type of *TP53* somatic mutations in ECs by performing a re-analysis of the publicly available data from The Cancer Genome Atlas (TCGA). A total of 228 EECs (n=186) and SECs (n=42) from TCGA dataset, for which an integrated genomic characterization was performed, were interrogated for the presence and type of *TP53* mutation, and for mutations in genes frequently mutated in ECs. *TP53* mutations were found in 15% of EECs and 88% SECs, and in 91% of copy-number high and 35% of POLE integrative genomic subtypes. In addition to differences in prevalence, variation in the type and pattern of *TP53* mutations were observed between histologic types and between the integrative genomic subtypes. *TP53* hotspot mutations were significantly more frequently found in SECs (46%) than in EECs (15%). *TP53*-mutant EECs significantly more frequently harbored a co-occurring *PTEN* mutation than *TP53*-mutant SECs. Finally, a subset of *TP53*-mutant ECs (22%) was found to harbor frameshift or nonsense mutations. Given that nonsense and frameshift *TP53* mutations result in distinct p53 immunohistochemical results that require careful interpretation, and that EECs and SECs display different patterns, types and distributions of *TP53* mutations, the use of the *TP53*/p53 status alone for the differential diagnosis of EECs and SECs may not be sufficient.

Keywords

endometrial cancer; *TP53* mutation; hotspot mutation; histologic subtype; genomic subtype

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare in relation to this study.

INTRODUCTION

Endometrial cancer (EC) is the most common malignant disease of the female genital system in North America, and comprises several histologic subtypes with distinct clinical behavior (1,2). Over the last decade it has become increasingly apparent that ECs are a heterogeneous group of tumors not only in terms of histology, biology, and clinical behavior, but also with respect to their genetic make-up (2-6). Endometrioid (EECs) and serous endometrial carcinomas (SECs), the two most common histologic EC subtypes, were found by candidate gene studies to be characterized by mutations affecting *PTEN*, *ARID1A*, *PIK3CA*, *KRAS* and *CTNNB1* (EECs) and by *TP53, PPP2R1A* and *FBXW7* mutations (SECs)(5,6). The Cancer Genome Atlas (TCGA) has recently reported a comprehensive genomic and transcriptomic analysis of EEC, SECs, and mixed endometrioid and serous carcinomas (4). On the basis of integration of mutation spectra defined by whole exome massively parallel sequencing analysis, copy number alterations and microsatellite instability, ECs were categorized into four genomic subtypes: POLE (ultramutated) tumors, microsatellite-instable (hypermutated) tumors (MSI), copy-number low (endometrioid) tumors (CN-low), and copy-number high (serous-like) tumors (CN-high). The latter genomic subtype of ECs, akin to serous carcinomas of the uterus, high-grade serous carcinomas of the ovary and basal-like breast cancers (4,7,8), displays recurrent mutations affecting *TP53*.

TP53 is the most commonly mutated gene in human cancers and alterations have been found in >50% of all human tumors (9,10). Whilst tumor suppressor genes are commonly inactivated by frameshift or nonsense mutations, the majority of mutations affecting *TP53* in human tumors are missense, and primarily affect the DNA-binding domain of the protein (exons 5-8). In fact, a number of so-called mutation hotspots have been identified, and approximately a third of all missense mutations affect the amino acid residues R175, G245, R248, R249, R273 and R282 (10-12). Mutant p53 proteins mostly lose their tumor suppressive functions, and may exert dominant-negative activities, but may also gain new oncogenic properties (10,11,13).

With the availability of complete sequencing of the entire coding region of *TP53* in multiple tumor types through large-scale massively parallel sequencing endeavors, it has become apparent that the frequency and spectrum of *TP53* mutations may differ according to tumor type and to molecular subtypes. For instance, in breast cancer, *TP53* mutations are found in approximately 85% of basal-like breast cancers, and in these tumors, a substantial proportion of the mutations are truncating or frameshift; on the other hand, luminal breast cancers harbor *TP53* mutations in a minority of lesions and these are predominantly missense mutations (7). Differences in the type of *TP53* mutations have been shown to have important consequences in the assessment of p53 by immunohistochemical analysis; whilst *TP53* missense mutations largely result in detectable p53 overexpression by immunohistochemistry, truncating and frameshift mutations often result in a negative immunohistochemical result (14,15).

Given the existence of multiple histologic and molecular subtypes of EC and the fact that approximately 25% of all ECs were found to harbor *TP53* mutations (4), we sought to define

the frequency, pattern and type of *TP53* somatic mutations in EC, and to determine whether the pattern of *TP53* mutations would vary according to the histologic or genomic subtypes of the disease. We performed a re-analysis of the EC TCGA dataset, and observed that not only the *TP53* mutation frequency (4), but also the *TP53* mutation spectrum is histologic subtype and genomic subtype-specific. In addition, our analysis suggests that *TP53* mutational or p53 immunohistochemical analyses may not be sufficient for the distinction between SECs and International Federation of Gynecology and Obstetrics (FIGO) grade 3 EECs, but should be combined with other immunohistochemical markers and/or a small set of genes frequently mutated in EECs and SECs.

MATERIALS AND METHODS

Patient selection

Clinico-pathologic data from endometrial cancers, including information on the four integrated genomic classes, were retrieved from the TCGA data portal (https://tcga-data.nci.nih.gov/docs/publications/ucec_2013/; file "Key Clinical Data")(4). From the 232 ECs for which an integrated genomic characterization was performed, we selected tumors of endometrioid (n=186) and serous (n=42) histologic subtypes (n=228 total). Mixed endometrial cancers (n=4) were excluded from this study. These publicly available data were interrogated for the presence and type of *TP53* mutations using cBioPortal (http://www.cbioportal.org; accessed January 2015)(16). All patients from the TCGA dataset had received no prior systemic treatment for their disease (4).

Histologic and genomic stratification

ECs were classified according to histologic type (i.e. endometrioid vs serous carcinomas), FIGO grade, and the four genomic subtypes as described by the TCGA (4), namely POLE (ultramutated), MSI (hypermutated), CN-low (endometrioid), and CN-high (serous-like). In addition to the presence and type of *TP53* mutations, also the presence of *ARID1A*, *FBXW7*, *PPP2R1A* and *PTEN* mutations was assessed and visualized using cBioPortal (http://www.cbioportal.org; accessed January 2015)(16).

Classification of *TP53* mutations

The *TP53* mutations identified were classified according to the predicted effect on protein function using the IARC *TP53* database (http://p53.iarc.fr; version R17, November 2013) (17). *TP53* mutations were further stratified according to i) mutation type, including single nucleotide missense mutations and other mutations (i.e. splice-site, nonsense, in-frame and frameshift), ii) the protein domain targeted by mutations, including the DNA-binding motif and outside DNA-binding motif, and iii) the functional effect, including hotspot (i.e. R175, G245, R248, R249, R273 and R282) mutations, as previously described (9,10,17-19). Mutation diagrams ("lollipop plots") were obtained from cBioPortal (www.cBioPortal.org) (16) and manually curated.

Statistical analysis

All statistical analyses were performed using the SPSS statistical software package (IBM SPSS, Version 21, IBM). Two-tailed Fisher's exact or Chi-square tests were employed for

comparisons between groups. Overall survival was expressed as the number of months from diagnosis to death (file “Key Clinical Data” (4)). Cumulative survival probabilities were calculated using the Kaplan–Meier method. Differences between survival rates were tested with the log-rank test (SPSS version 21; IBM). A p-value<0.05 was considered statistically significant.

RESULTS

TP53 mutational status in ECs

Of the 228 SECs and EECs included in this study, 64 (28%) harbored a mutation in *TP53*. Four EECs harbored multiple *TP53* mutations; in these cases, we have observed a combination of multiple missense mutations (n=2), a missense and a frameshift mutation (n=1), or a missense and a nonsense mutation (n=1; Supplemental Table 1). For the subsequent analyses reported in this study, the two cases harboring a missense and a frameshift mutation or a missense and a nonsense mutation, were classified as *TP53*-mutant harboring a frameshift or a nonsense mutation, respectively, as these EECs were of ultramutated POLE genomic subtype (see below) and the *TP53* missense mutations were rare non-hotpot mutations.

A comparative analysis of *TP53* mutations according to histologic types revealed that SECs are significantly more frequently *TP53*-mutant than EECs (88% vs 15%, respectively; Fisher’s exact test p<0.0001; Table 1) (4-6,20). As expected, *TP53*-mutant ECs had a significantly worse overall survival than *TP53* wild-type cancers (p=0.035; Supplemental Fig. 1A) (21,22). Within EECs, the frequency of *TP53* mutations was significantly associated with tumor grade, with 3% (2/68), 11% (8/72) and 37% (17/46) of FIGO grade 1, grade 2, and grade 3 cancers harboring *TP53* mutations, respectively (Chi-squared p<0.0001; Tables 1 and 2; Supplemental Table 2) (23). The presence of *TP53* mutations in EECs, however, was not significantly associated with outcome in the series analyzed (p>0.1; Supplemental Fig. 1B).

Given that *TP53* mutations were relatively uncommon in EECs, and to understand the spectrum of *TP53* mutations in ECs, we have focused only on *TP53*-mutant tumors for further analyses. Although no correlation between the type of *TP53* mutation (i.e. missense, frameshift or nonsense) and tumor type was observed (Chi-squared p>0.1), we have found that SECs significantly more frequently harbored *TP53* hotspot mutations than EECs (17/37, 46% vs 4/27, 15%, respectively, Fisher’s exact test p<0.05; Fig. 1A, Table 2).

Importantly, in the 64 *TP53*-mutant ECs identified in this dataset, *TP53* frameshift or nonsense mutations were present in 14 cases (22%, Table 2), of which only two also displayed a missense mutation (Supplemental Table 1). Importantly, as somatic frameshift or nonsense mutations do not result in a p53 protein expression stabilization (15), in up to 22% of ECs (or in up to 19% if the two cases with both missense and truncating or frameshift mutations were excluded) these *TP53* somatic mutations may theoretically not be detected as p53 overexpression by immunohistochemical analysis. Instead, frameshift and nonsense *TP53* mutations are associated with a complete absence of p53 immunoreactivity, also

referred to as ‘null pattern’, which requires careful interpretation of the immunohistochemical results (24-27).

Taken together, these observations demonstrate that *TP53* mutations, although more frequently found in SECs and FIGO grade 3 EECs, are not restricted to these types of ECs, as a subset of FIGO grade 1 and 2 EECs may also harbor mutations affecting *TP53*. Furthermore, the spectrum of *TP53* mutations differs between SECs and EECs, with the former displaying a significant enrichment for hotspot mutations.

TP53 mutation status according to integrative genomic subtypes

We next sought to define the spectrum of *TP53* somatic mutations according to the integrative genomic subtypes of ECs. *TP53* somatic mutations were found to be significantly more frequent in CN-high (91%) and POLE (35%) than in MSI (8%) and CN-low ECs (1%, Chi-squared $p<0.0001$; Table 1). Consistent with the ultra-high mutation rate of POLE ECs, all cases harboring more than one *TP53* somatic mutation were of POLE subtype, and frameshift, nonsense and splice-site mutations were restricted to CN-high and POLE cancers (Table 3; Supplemental Table 1).

The spectrum of *TP53* somatic mutations in CN-high appears to be somewhat different from that of other subtypes of ECs, in that CN-high cancers harbored mutations affecting hotspot residues numerically more frequently (20/52, 38% *TP53*-mutant CN-high cancers vs 1/12, 8% *TP53*-mutant non-CN-high cancers), however this was not statistically significant (Fisher’s exact test $p=0.084$; Table 3). Given that the subset of CN-high ECs comprise both SECs and EECs, we next sought to define whether there would be differences in the frequency, type and pattern of *TP53* mutations in CN-high ECs. Notably, SECs and EECs of CN-high genomic subtype had similar frequencies of *TP53* somatic mutations, and also a similar distribution of missense and hotspot mutations (Table 3; Supplemental Table 3).

Taken together, our results demonstrate that the vast majority of CN-high ECs are *TP53*-mutant and that the spectrum of *TP53* mutations is similar in SECs and EECs of CN-high subtype. Importantly, however, *TP53* mutations cannot be employed as a defining feature of this subtype, given that up to a third of POLE and 8% of MSI (endometrioid) cancers are also *TP53*-mutant, and that 10% of CN-high SECs and 6% of CN-high EECs are *TP53* wild-type (Supplemental Table 3).

Associations between highly recurrently mutated genes in ECs and *TP53* mutational status

The TCGA study identified several mutated genes that were characteristic of the different genomic subtypes of ECs. Epistatic interactions between genes and mutations may not only determine the evolutionary properties of cancers but may also affect their fitness and response to therapies (28). Given that the frequency and type of *TP53* mutations would not provide sufficient information to differentiate between FIGO grade 3 EECs and SECs, we sought to define whether mutations affecting genes preferentially mutated in EECs or SECs would provide additional information.

Mutations affecting *ARID1A* and *PTEN* have been reported to be characteristic of EECs, whereas SECs show an enrichment for mutations affecting *FBXW7* and *PPP2R1A*

(4,6,20,23,29-32). As expected, in this dataset of 228 EECs and SECs, *ARID1A* and *PTEN* were significantly more frequently mutated in EECs (*ARID1A*, 39% EEC vs 10% SECs; *PTEN*, 78% EECs vs 2% SECs; Fisher's exact test p<0.001), whereas *FBXW7* and *PPP2R1A* were significantly more frequently mutated in SECs (*FBXW7*, 12% EECs vs 33% SECs; *PPP2R1A*, 7% EECs vs 26% SECs; Fisher's exact test p<0.005; Supplemental Table 4).

The associations between *TP53* somatic mutations and mutations affecting *ARID1A, FBXW7, PPP2R1A* and *PTEN* varied according to histologic type. In EECs, although no differences in the prevalence of *FBXW7* and *PPP2R1A* mutations were identified, a significant inverse association between *TP53* somatic mutations and *ARID1A* somatic mutations was observed, where *ARID1A* somatic mutations were found in 15% and 43% of the *TP53*-mutant and wild-type EECs, respectively (Fisher's exact test p<0.001; Table 1, Fig. 1B, Supplemental Table 4). We found that *ARID1A* mutations were particularly frequent in EECs of MSI (endometrioid) integrative subtype (40%), a subtype that generally harbored few *TP53* mutations (Table 1). *PTEN* somatic mutations were significantly less frequently found in *TP53*-mutant (63%) than in *TP53* wild-type EECs (81%; Fisher's exact test p<0.05; Fig. 1B; Supplemental Table 4). Given that 88% of SECs harbored a *TP53* somatic mutation, no associations between *TP53* mutations and mutations affecting *ARID1A, FBXW7, PPP2R1A* and *PTEN* were identified.

Within the subset of *TP53*-mutant ECs, a significant association between histologic type and *PTEN* mutations was identified (Table 1). Whilst 63% of all *TP53*-mutant EECs displayed a *PTEN* somatic mutation, only 3% of *TP53*-mutant SECs harbored somatic mutations affecting this gene (Fisher's exact test p<0.0001; Supplemental Table 4). When focusing on high-grade ECs of CN-high subtype only, however, *PTEN* somatic mutations were observed at similar frequencies in CN-high *TP53*-mutant FIGO grade 3 EECs (2/9, 22%) and CN-high *TP53*-mutant SECs (3/37, 8%; Fisher's exact test p=0.2484; Supplemental Table 3). Conversely, FIGO grade 3 *TP53* wild-type EECs were statistically significantly more likely to harbor a *PTEN* mutation (22/29, 76%) than TP53 wild-type SECs (0/5, 0%; Fisher's exact test, p=0.0028), providing evidence to suggest that the analysis of PTEN in high-grade ECs may help in discriminating FIGO grade 3 *TP53* wild-type EECs from *TP53* wild-type SECs (33-36). It should be noted, however, that the distinction between SEC and FIGO grade 3 EEC is challenging, even among expert gynecologic pathologists (33,37).

We next sought to define whether the information provided by the mutational status of *FBXW7* or *PPP2R1A* genes, which are significantly more frequently targeted by mutations in SECs than in EECs (4,29) (Supplemental Table 4), would be useful in the discrimination of FIGO grade 3 EECs and SECs. We found that 20/37 (54%) of *TP53*-mutant SECs harbored a mutation affecting *FBXW7* and/or *PPP2R1A*, as compared to 2/17 (12%) of FIGO grade 3 *TP53*-mutant EECs (Fisher's exact test p=0.0062; Fig. 1B; Supplemental Table 4). In the remaining cases, *TP53*-mutant *FBXW7/PPP2R1A* wild-type SECs were significantly less frequently affected by *PTEN* mutations (1/17, 6%) as compared to FIGO grade 3 *TP53*-mutant *FBXW7/PPP2R1A* wild-type EECs (8/15, 53%, Fisher's exact test p=0.0049).

Spectrum of *TP53* mutations in ECs

Given that POLE cancers often harbored more than one *TP53* somatic mutation, we next investigated the distribution of the *TP53* mutations according to exons of the *TP53* gene. In this dataset, 69 *TP53* mutations were found in SECs and EECs (Table 4).

When we assessed the single base substitutions, we observed that in EECs, these were predominantly T>C (8/28, 28%) and G>A (8/28, 28%) transitions, followed by C>T (5/28, 17%) transitions and C>A transversions (3/28, 10%). By contrast, in SECs, the most common single base substitutions were C>T transitions (12/35, 34%), followed by G>A transitions (11/35, 31.5%) and C>A transversions (3/35, 8.5%). EECs significantly more often harbored T>C transitions than SECs (EECs 8/28, SECs 2/35; Fisher's exact test p=0.0179; data not shown).

Consistent with the notion that *TP53* mutations preferentially affect exons 5-8 (10-12), in this dataset of ECs, *TP53* mutations were found to primarily affect exon 7 (25/69, 36%) and exon 8 (17/69, 25%) of the *TP53* gene, whereas exons 4, 9 and 10 harbored the lowest number of mutations (n=2, n=1 and n=2, respectively; Table 4). Interestingly, whilst the number of *TP53* mutations affecting exons 7 and 8 were similar between EECs and SECs (exon 7: 11/32, 34% EECs; 14/37, 38%, SECs, Fisher's exact test p=0.806; exon 8: 5/32, 5% EECs; 12/37, 32%, SECs, Fisher's exact test p=0.161), we observed that EECs statistically significantly more frequently harbored *TP53* exon 6 mutations than SECs (exon 6: 8/32, 25% EECs; 2/37, 5% SECs; Fisher's exact test, p=0.037; Table 4). In fact, mutations affecting exon 6 of *TP53* were significantly more frequently found in POLE cancers than in other genomic subtypes (36% of the *TP53* mutations affected exon 6 in POLE cancers, whereas only 12% of *TP53* mutations affected exon 6 in the other integrative genomic subtypes, Fisher's exact test p-value<0.05).

DISCUSSION

Here we demonstrate that *TP53* mutations are present in 28% of the 228 ECs analyzed, and that although the vast majority of SECs harbor *TP53* mutations, up to 15% of all EECs are also *TP53*-mutant. Within EECs, we have observed that *TP53* mutations are more frequently found in tumors of FIGO grade 3, and of CN-high and POLE integrative genomic subtypes (4). Given that up to a third of POLE and 8% of MSI (endometrioid) cancers are *TP53*-mutant, and that 10% of CN-high SECs and 6% of CN-high EECs are *TP53* wild-type, *TP53* mutations cannot be employed as a defining feature of ECs of CN-high (serous-like) integrative subtype or the discrimination between EECs and SECs of this genomic subgroup. The 12% of SECs in this series not harboring *TP53* mutations were of CN-high (4/5) or CN-low (1/5) integrative genomic subtypes, and four cases harbored a median of 690 (range 238-1133) gene copy number alterations and 41 (range 30-43) non-synonymous somatic mutations (Supplemental Table 5). One *TP53* wild-type SEC, however, harbored 1,324 non-synonymous mutations and few copy number altered genes (n=5), and mutations characteristic for both SECs and EECs (Supplemental Table 5).

The type of *TP53* mutations present in ECs varied according to histologic and molecular subtypes. *TP53* hotspot mutations were significantly more frequently found in SECs than in

EECs. These hotspot mutations affect residues that are either involved in DNA binding (e.g., R248 and R273) or in supporting the structure/conformation of the DNA-binding surface (e.g., R175, G245, R249, R282)(12,38). There is burgeoning evidence to demonstrate that all *TP53* hotspot mutations are in fact pathogenic and result in loss of p53 protein activity, whereas this is less clear for missense mutations that affect codons other than the non-hotspot residues (38,39). In this study, 46% of the *TP53* mutations in SECs affected hotspot residues, whereas in EECs hotspot mutations were significantly less frequent (15%) and numerically less frequent in CN-high EECs than in CN-high SECs (20% vs 46%, respectively, Fisher's exact test p-value=0.084). These observations suggest that even when present in EECs, the biological impact of *TP53* mutations may differ between EECs and SECs.

Data from breast cancer studies have demonstrated that *TP53* mutations are found in all molecular subtypes, but differ in their frequency, type and pattern according to the molecular subtypes (7,18). Our results demonstrate that akin to breast cancers, the frequency and type of *TP53* mutations also varies according to the EC integrative genomic subtypes. Unlike breast cancers, however, where the basal-like group is the most frequently mutated and is enriched for frameshift and nonsense mutations (7,18), in ECs, the CN-high genomic subtype is the most frequently mutated but displays an enrichment for missense mutations affecting hotspot residues. In fact, a re-analysis of the TCGA ovarian cancer study revealed that the repertoire of *TP53* mutations in SECs is more similar to that of high-grade serous ovarian carcinomas than to that of basal-like breast cancers (4,7,8), further supporting that although there are similarities between these tumors, important differences are also observed.

Although the protein product of *TP53* harboring missense mutations has been shown to have a longer half-life and, therefore, amenable to immunohistochemical detection, cases harboring *TP53* frameshift and nonsense mutations have been shown to frequently yield negative immunohistochemical results (i.e. p53 overexpression cannot be detected)(15,40). Immunohistochemical analysis of p53, however, has been suggested to provide ancillary information for accurate diagnosis of SECs (6). Our results demonstrate that approximately one fifth of all *TP53*-mutant ECs harbor frameshift or nonsense mutations. In these cases, careful interpretation of the p53 immunohistochemical results is essential as frameshift and nonsense *TP53* mutations lead to a complete lack of p53 protein expression ('null pattern') (24-27), which may be misinterpreted as wild-type p53 expression pattern. Further studies investigating the type of *TP53* mutation and the respective p53 protein expression patterns by immunohistochemistry using distinct p53 antibody clones are warranted.

Finally, we sought to define whether sequencing analysis of additional genes highly mutated in ECs may assist in the differentiation between SECs and high-grade EECs. In fact, we have observed that within the group of *TP53*-mutant ECs, somatic mutations affecting *PTEN* are significantly more frequently found in EECs than in SECs. Importantly, however, not all high-grade EECs, and in particular only a small subset of FIGO grade 3 EECs of CN-high integrative subtype, harbor *PTEN* somatic mutations and, therefore, would have retained PTEN expression by immunohistochemical analysis, given the strong correlation between *PTEN* somatic mutations and lack of protein expression in ECs (41). We further

demonstrated that the mutational analysis of *FBXW7*, *PPP2R1A* and *PTEN* together with the assessment of histologic features associated with microsatellite instable (MSI) ECs and ECs harboring *POLE* hotspot mutations (i.e. enrichment in tumor-infiltrating lymphocytes and/or peri-tumoral lymphocytes (42,43)) and the immunohistochemical analysis of the DNA mismatch repair markers (MMR) (4,43), may aid in the distinction of between SECs and FIGO grade 3 EECs (Fig. 2). In the sporadic ECs analyzed by TCGA (4), high-level MSI was associated with *MLH1* hypermethylation and endometrioid histology in all cases studied, thus the DNA MMR testing may be restricted to MLH1 immunohistochemical analysis, followed by *MLH1* methylation analysis if MLH1 protein expression is absent (Fig. 2). It should be noted however, that in the setting of Lynch syndrome, 14-35% of ECs are not of endometrioid histology (44,45), hence the proposed scheme is only applicable for sporadic ECs. Studies to validate the proposed scheme in independent datasets are warranted.

It is important to note that genetic alterations affecting *PTEN* and *FBXW7* may not only be useful for the histologic typing of high-grade *TP53*-mutant ECs (Fig. 2), but may also have therapeutic implications. Given that the vast majority of ECs have been found to harbor somatic genetic alterations in the PI3K/AKT/mTOR pathway (4), inhibition of this pathway is of great therapeutic interest (46). In particular, there is pre-clinical evidence to suggest that ECs harboring *PIK3CA* or *PTEN* mutations may be sensitive to inhibitors targeting different components of the PI3K/AKT/mTOR pathway (47-51). *FBXW7* is a tumor suppressor gene, and its protein product FBXW7 promotes the ubiquitination and degradation of numerous oncoproteins (52). Histone deacetylase (HDAC) inhibition (53) or targeting of FBXW7 regulators in *FBXW7*-mutant cancers, such as NOTCH1 (54) or mTOR (55,56), may also have potential for therapeutic interventions in ECs harboring *FBXW7* inactivating mutations.

The genomic subtypes of ECs as proposed by TCGA, which are defined through gene copy number and exome-wide mutational analysis, have yet to be incorporated into clinical practice. It is likely that the integration of both genomics and histopathology may lead to a classification system of ECs that helps define biologically and clinically relevant subsets of the disease, and may facilitate development of therapies tailored to specific histologic and genomic subgroups (6) as outlined above. Recent studies have confirmed that ECs of POLE ultramutated genomic subtype have a good prognosis (57,58), despite these tumors commonly being of high grade and frequently harboring *TP53* mutations (42). This POLE subgroup of ECs is currently only reliably identified by sequencing of the *POLE* gene. In this context it is worth mentioning that in other disease types the integration of histologic and molecular information is already being performed. For example, neuropathologists recently reported on consensus guidelines that will incorporate molecular information in the next World Health Organization (WHO) classification (59), where distinct tumor entities will be derived from an “integrated” diagnosis, seeking to define biologically and clinically uniform groups precisely and objectively on the basis of a combination of molecular information, histologic features and WHO grade (59). The therapy of patients with high-grade ECs is currently primarily based on clinical parameters rather than on the biological characteristics of the tumors, and the incorporation of molecular features of these cancers

will be essential for the realization of the potentials of precision medicine for patients with high-grade ECs.

In conclusion, the type and pattern of *TP53* mutations varies according to histologic and genomic subtypes of EC. Importantly, *TP53* mutations are not restricted to SECs, FIGO grade 3 and/or CN-high lesions. Given that a subset of *TP53*-mutant ECs harbor nonsense or frameshift mutations, for which interpretation of immunohistochemistry results may be challenging, and that EECs and SECs display different patterns, types and distributions of *TP53* mutations, p53 immunohistochemical analysis alone may not be sufficient for the differential diagnosis of EEC and SECs, and a panel comprising additional genes, including *FBXW7*, *PPP2R1A* and *PTEN*, may provide further diagnostic accuracy in challenging cases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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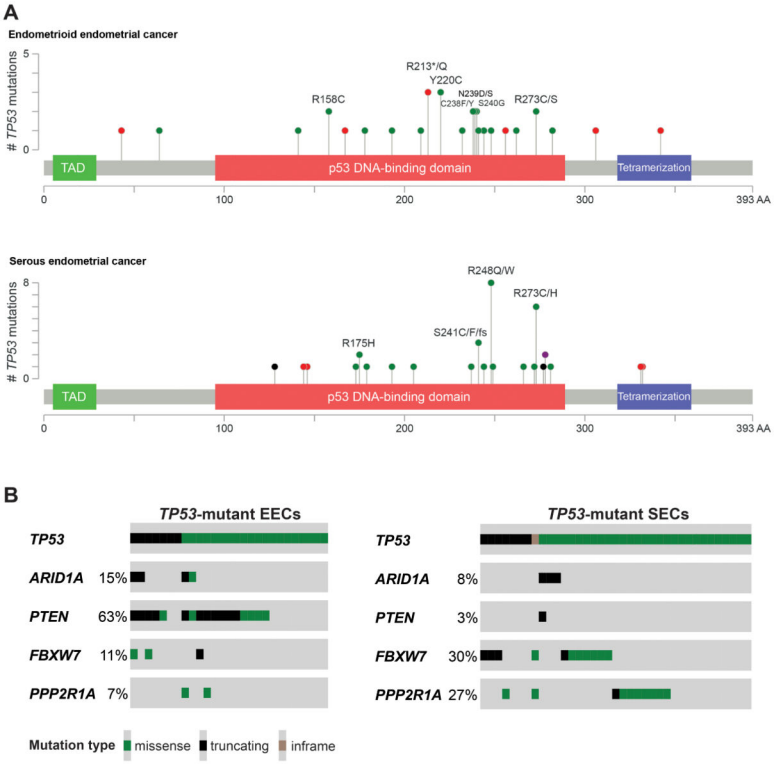

Fig. 1. Distribution and spectrum of *TP53* mutations in endometrial cancer

(A) Distribution and spectrum of *TP53* mutations in endometrioid endometrial cancer (top) and serous endometrial cancer (bottom). Diagrams represent the protein domains of p53 encoded by *TP53*. The presence of a mutation is shown on the x-axis ('lollipop'), the frequency of mutations is shown on the y-axis. Missense mutations are presented as green circles, truncating mutations (i.e. nonsense, frameshift, splice-site) are depicted in red circles, in-frame insertions and deletions are shown in black circles, and circles colored in purple indicate residues affected by different types of mutation at the same proportion. Plots were generated using cBioPortal (www.cBioPortal.org) and manually curated. Note that mutations affecting the hotspots R175, R248 and R273 are more frequent in serous than in endometrioid endometrial cancers. (B) Prevalence of *ARID1A*, *PTEN*, *FBXW7* and *PPP2R1A* mutations in *TP53*-mutant endometrioid endometrial cancers (EECs; left), and prevalence of *ARID1A*, *PTEN*, *FBXW7* and *PPP2R1A* mutations in *TP53*-mutant serous endometrial cancers (SECs; right). Mutation types/ color-codes as depicted in the legend. Plots were generated using cBioPortal (www.cBioPortal.org) and manually curated.

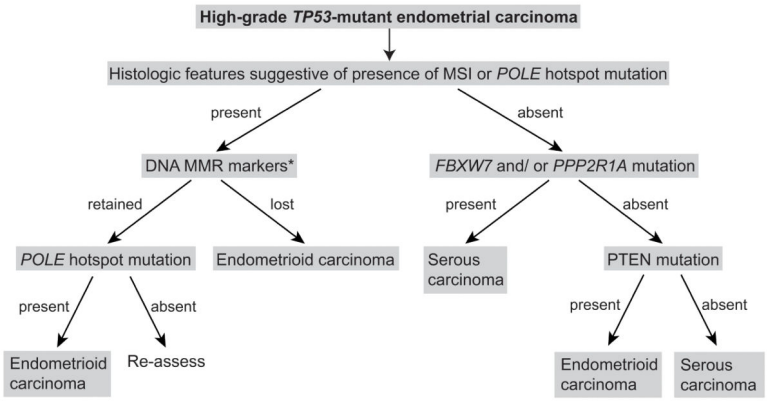

Fig. 2. A combination of immunohistochemical and mutational analysis may aid in the distinction of sporadic high-grade *TP53*-mutant endometrial carcinomas of serous endometrial and FIGO grade 3 endometrioid histologic subtypes

Histologic features characteristic of MSI endometrial cancers or endometrial carcinomas harboring *POLE* hotspot mutations include enrichment in tumor-infiltrating lymphocytes and/or peri-tumoral lymphocytes, among others. *DNA MMR markers may be limited to MLH1 immunohistochemistry and/or *MLH1* promoter methylation analysis. DNA MMR, DNA mismatch repair; IHC, immunohistochemistry; MSI, microsatellite instability.

Table 1

TP53 mutational status according to clinico-pathologic characteristics and co-occurrence with somatic mutations in genes frequently altered in endometrial carcinomas.

		Total (n)	*TP53* gene status		p-value
			Wild-type (n=164)	Mutant (n=64)	
Histologic type	Endometrioid	186	159 (85%)	27 (15%)	<0.0001 *
	Serous	42	5 (12%)	37 (88%)	
FIGO grade	Grade 1	68	66 (97%)	2 (3%)	<0.0001 **
	Grade 2	72	64 (89%)	8 (11%)	
	Grade 3	88	34 (39%)	54 (61%)	
Integrative genomic subtype	CN-high	57	5 (9%)	52 (91%)	<0.0001 **
	CN-low	89	88 (99%)	1 (1%)	
	MSI	65	60 (92%)	5 (8%)	
	POLE	17	11 (65%)	6 (35%)	
ARID1A gene status	Wild-type	151	94 (62%)	57 (38%)	<0.0001 *
	Mutant	77	70 (91%)	7 (9%)	
FBXW7 gene status	Wild-type	191	141 (74%)	50 (26%)	0.1641 *
	Mutant	37	23 (62%)	14 (38%)	
PPP2R1A gene status	Wild-type	204	152 (75%)	52 (25%)	0.0162 *
	Mutant	24	12 (50%)	12 (50%)	
PTEN gene status	Wild-type	81	35 (43%)	46 (57%)	<0.0001 *
	Mutant	147	129 (88%)	18 (12%)	

CN-high, copy-number high (serous-like) integrative genomic subtype; CN-low, copy-number low (endometrioid) integrative genomic subtype; MSI, microsatellite instable (hypermutated) integrative genomic subtype; n, number of cases; POLE, POLE (ultramutated) integrative genomic subtype.

* Fisher's exact test p-value;

** Chi-square test p-value

Table 2

Clinico-pathologic features, distribution of *TP53* mutations and mutations in genes recurrently altered in endometrial cancers in *TP53*-mutant endometrioid and serous endometrial carcinomas.

		Total (n)	Histologic type		p-value
			Endometrioid (n=27)	Serous (n=37)	
Tumor grade	Grade 1	2	2	0	0.0002 **
	Grade 2	8	8	0	
	Grade 3	54	17	37	
Integrative genomic subtype	CN-high	52	15	37	0.0002 **
	CN-low	1	1	0	
	MSI	5	5	0	
	POLE	6	6	0	
Type of *TP53* mutation	Frameshift	7	3	4	0.7030 **
	Missense	49	20	29	
	Nonsense	7	4	3	
	Splice-site	1	0	1	
Hotspot *TP53* mutation	No	43	23	20	0.0144 *
	Yes	21	4	17	
ARID1A gene status	Wild-type	57	23	34	0.4427 *
	Mutant	7	4	3	
FBXW7 gene status	Wild-type	50	24	26	0.1247 *
	Mutant	14	3	11	
PPP2R1A gene status	Wild-type	52	25	27	0.0578 *
	Mutant	12	2	10	
PTEN gene status	Wild-type	46	10	36	<0.0001 *
	Mutant	18	17	1	

CN-high, copy-number high (serous-like) integrative genomic subtype; CN-low, copy-number low (endometrioid) integrative genomic subtype; MSI, microsatellite instable (hypermutated) integrative genomic subtype; n, number of *TP53*-mutant cases; POLE, POLE (ultramutated) integrative genomic subtype. Hotspot *TP53* mutations include R175, G245, R248, R249, R273 and R282.

* Fisher's exact test p-value;

** Chi-square test p-value.

Table 3

Clinico-pathologic features, distribution of *TP53* mutations and mutations in genes recurrently altered in endometrial cancers in *TP53*-mutant endometrial carcinomas classified according to integrative genomic subtypes.

		Total (n)	Integrative genomic subtype				p-value
			CN-high (n)	CN-low (n)	MSI (n)	POLE (n)	
Tumor grade	Grade 1	2	0	0	0	2	<0.0001 *
	Grade 2	8	6	1	1	0	
	Grade 3	54	46	0	4	4	
Histologic type	Endometrioid	27	15	1	5	6	<0.0001 *
	Serous	37	37	0	0	0	
Type of *TP53* mutation	Frameshift	7	6	0	0	1	0.1844 **
	Missense	49	41	1	5	2	
	Nonsense	7	4	0	0	3	
	Splice-site	1	1	0	0	0	
Hotspot *TP53* mutation	No	43	32	1	5	5	0.2545 *
	Yes	21	20	0	0	1	
Cases with multiple *TP53* mutations	No	60	52	1	5	2	<0.0001 *
	Yes	4	0	0	0	4	
ARID1A gene status	Wild-type	57	49	1	4	3	0.0140 *
	Mutant	7	3	0	1	3	
FBXW7 gene status	Wild-type	50	41	1	5	3	0.2631 *
	Mutant	14	11	0	0	3	
PPP2R1A gene status	Wild-type	52	41	1	5	5	0.85549 *
	Mutant	12	11	0	0	1	
PTEN gene status	Wild-type	46	46	0	0	0	<0.0001 *
	Mutant	18	6	1	5	6	

CN-high, copy-number high (serous-like) integrative genomic subtype; CN-low, copy-number low (endometrioid) integrative genomic subtype; MSI, microsatellite instable (hypermutated) integrative genomic subtype; n, number of *TP53*-mutant cases; POLE, POLE (ultramutated) integrative genomic subtype. Hotspot *TP53* mutations include R175, G245, R248, R249, R273 and R282.

* Fisher's exact test p-value;

** Chi-square test p-value.

Table 4

Spectrum of *TP53* mutations according to histologic types and integrative genomic subtypes.

		Total (n)	Histologic type		p-value	Integrative genomic subtype				p-value
			Endometrioid (n)	Serous (n)		CN-high (n)	CN-low (n)	MSI (n)	POLE (n)	
Type of *TP53* mutation	Frameshift	8	4	4	0.6063 **	6	0	0	2	0.3111 **
	Missense	52	23	29		41	1	5	5	
	Nonsense	8	5	3		4	0	0	4	
	Splice-site	1	0	1		1	0	0	0	
Exon	Exon 4	2	2	0	0.0366 ***	1	0	0	1	0.1311 ***
	Exon 5	12	5	7		10	0	1	1	
	Exon 6	10	8	2		6	0	0	4	
	Exon 7	25	11	14		17	1	4	3	
	Exon 8	17	5	12		16	0	0	1	
	Exon 9	1	0	1		1	0	0	0	
	Exon 10	2	1	1		1	0	0	1	
Hotspot	No	48	28	20	0.0036 *	32	1	5	10	0.08678 *
	Yes	21	4	17		20	0	0	1	
Multiple *TP53* mutations	No	60	23	37	0.0276 *	52	1	5	2	<0.0001 *
	Yes	4	4	0		0	0	0	4	

CN-high, copy-number high (serous-like) integrative genomic subtype; CN-low, copy-number low (endometrioid) integrative genomic subtype; MSI, microsatellite instable (hypermutated) integrative genomic subtype; n, number; POLE, POLE (ultramutated) integrative genomic subtype.

* Fisher's exact test p-value;

** Chi-square test p-value;

*** Fisher's exact test p-value comparing exon 6 vs mutations affecting other exons.