

Clinical Relevance of Oncogenic Driver Mutations Identified in Endometrial Carcinoma

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Abstract

Background: Endometrial carcinoma is a clinically heterogeneous disease characterized by a number of different histological subtypes, and its heterogeneity may be involved in the accumulation of multiple genetic alterations. The aim of this work is to investigate a comprehensive mutational profile of endometrial carcinoma tumors, and examine the correlations with somatic mutations and clinicopathological features or survival in endometrial carcinoma patients.

Methods: A total of 100 surgical tumors were obtained from endometrial carcinoma patients who had undergone surgery at Fukushima Medical University Hospital between 2013 and 2017. Genomic DNA samples extracted from fresh-frozen tissues were analyzed using the Ion AmpliSeq Cancer Hotspot Panel v2 Kit covering 50 tumor-related genes.

Results: Validated mutations were detected in 91 of the 100 tumors (91%) and identified in eight of the most frequently mutated genes, namely *PTEN* (57%), *PIK3CA* (51%) and *TP53* (30%) *KRAS* (23%), *CTNNB1* (21%), *FBFR2* (13%), *FBXW7* (10%) and *RB1* (9%). *PTEN* mutations correlated with young age (< 60), early-stage, endometrioid histology, non-recurrence and better overall survival. *CTNNB1* mutations correlated with young age, endometrioid histology and better overall survival. On the other hands, *TP53* mutations correlated with late-stage, non-endometrioid histology, high-grade, recurrence and worse overall survival. *FBWX7* mutations correlated with late-stage, vascular invasion and lymph node metastasis. *FGFR2* mutations correlated with deep ($\geq 1/2$) myometrial invasion. No statistically significant associations were found between clinicopathological characteristics or overall survival and *PIK3CA*, *KRAS* or *RB1* mutations were found.

Conclusion: Our study demonstrated that mutations in *PTEN* and *CTNNB1* could be predictive biomarkers for favorable outcome, while mutations in *TP53*, *FBXW7* and *FGFR2* could be those for adverse outcome. Our comprehensive mutational profile is useful for understanding and evaluating the molecular characteristics of endometrial carcinoma patients, and will lead to the establishment of novel treatment strategies that improve the survival of patients with endometrial carcinoma in the future.

Introduction

Endometrial carcinoma (EC) is the most frequent malignancy of the female genital tract, and the seventh most commonly occurring cancer in women worldwide, as of 2018 [1]. EC often produces symptoms, such as postmenopausal or abnormal genital bleeding, at a relatively early-stage, so the disease is generally diagnosed early. Although more than 75% of EC patients are stage I at the time of diagnosis, and the 5-year overall survival (OS) rate for women with early-stage EC exceeds 80%, 10–15% of all tumors have a recurrence [1, 2]. The stage, histological type, tumor grade, myometrial invasion (MI), vascular invasion (VI) and lymph node metastasis (LNM) at the time of treatment are defined as independent prognostic factors in patients with EC by the International Federation of Obstetrics and Gynecology (FIGO) [3]. However, to explain EC heterogeneity with these clinicopathological factors alone

is impossible [4]. Therefore, identification of new predictive markers for a high-risk phenotype would be very useful for selection of the most efficient therapies and the development of novel treatment modalities.

Recently, comprehensive molecular profiling of cancer using next-generation sequencing (NGS) approaches is increasingly used in oncology for diagnostic and therapeutic management decisions. Cancer-specific mutations, including single-nucleotide alterations and small insertions or deletions, are known to affect key driver genes early during tumorigenesis [5]. There are two types of EC: Type I EC (80–90%) characterized by an excess of estrogen is typically endometrioid endometrial carcinoma (EEC) with low-grade and has a favorable outcome. In contrast, type II EC (10–20%) is usually non-endometrioid endometrial carcinoma (NEEC) (serous carcinoma, clear cell carcinoma and malignant mixed mullerian tumor) with high-grade tumors and poor prognosis. In previous reports, frequent somatic mutations of *PTEN*, *CTNNB1*, *PIK3CA*, *ARID1A* and *KRAS* in type I EC, as well as those of *TP53* genes in type II EC, have been identified using whole exome and genome sequencing analysis [6, 7]. Although several studies have reported associations between each somatic mutation and clinicopathological characteristics of EC [8–10], there have been few studies focusing on a comprehensive somatic mutation analysis [6]. In the present study, we performed a comprehensive mutational profile in EC patients using a cancer panel, and examined whether somatic mutation status is associated with risk factors or survival.

Materials And Methods

Clinical samples

A total of 100 surgical tumors were obtained from EC patients who had undergone surgery at Fukushima Medical University Hospital between August 2013 and December 2017. The clinicopathological data for age, staging (FIGO 2008), histology, tumor grade (EEC), MI, VI, LNM, and recurrence was collected by operative reports, clinical notes and pathological reports. Histology was divided into EEC and NEEC. Adjuvant therapy was determined according to the physician's treatment strategy. The study design was approved by the ethics committee of Fukushima Medical University (No. 1953), and informed consent was obtained from all participants. All analyses were performed in accordance with the relevant guidelines and regulations.

Dna Extraction

Isolation and purification of genomic DNAs after extraction of RNAs from 100 fresh- frozen tumor samples were performed using ISOGEN reagent (Nippongene, Tokyo, Japan), according to the manufacturer's instructions. The concentration and quality of each DNA sample were assessed using NanoDrop One (ThermoFisher Scientific, Waltham, MA, USA).

Somatic Mutation Detection

The Ion Ampliseq Cancer Hotspot Panel v2 on the Ion Torrent platform was used to detect 2790 mutations in 50 oncogenes and tumor suppressor genes [11, 12]. In brief, 10 ng of genomic DNAs extracted from 100 frozen tumor samples were used to construct barcoded DNA libraries utilizing an Ion Ampliseq Library Kit 2.0 (Thermo Fisher Scientific). The obtained libraries were optimized using an Ion Library Equalizer Kit (Thermo Fisher Scientific), and then sequenced using an Ion Personal Genome Machine or Ion S5XL platform (Thermo Fisher Scientific). The sequencing reads were aligned to the reference genome build hg19, GRCh37, and converted into BAM files using Ion Torrent Suite software (Thermo Fisher Scientific). Sequence variants were then called using Ion Reporter 5.0 (Thermo Fisher Scientific), according to the manufacturer's instructions. The average sequencing depth reached at least 1500-fold per sample.

Statistical analysis.

The associations between somatic mutations and categorical variables, as well as between mutation rate or frequency and categorical variables, were evaluated using the chi-squared test or Mann-Whitney U Test. OS was evaluated as clinical outcomes. Survival distributions were calculated according to the Kaplan-Meier method, and statistical significance was determined using the log-rank test. Values of $P < 0.05$ were considered statistically significant. Statistical analysis of data was performed using SPSS version 25 software (SPSS, Inc., Chicago, IL, USA).

Results

Clinicopathological characteristics.

The clinicopathological characteristics of all the patients are shown in Table 1. A total of 100 EC patients who had been treated with surgery were enrolled in this study. The median age at diagnosis was 62.5 years (range, 32–89 years), and 62 (62%) of the patients with high recurrence risk or advanced stage underwent platinum-based chemotherapy postoperatively. Among the 100 tumors, 82 were EEC and 18 were NEEC (nine serous, six clear cell, two undifferentiated and one mixed carcinoma). After a median follow-up of 37 months (range, 1–76 months), 78 (78%) patients were alive without clinical evidence of tumor. Recurrence was identified during the follow-up period in 22 (22%) patients: six (6%) patients were alive with disease; sixteen (16%) patients died due to their tumor between postoperative months 1 and 42 (median, 7.5 months).

Table 1
Clinicopathological
characteristics of
patients with
endometrial
cancer.

Spectrum and frequency of mutations in EC.

In order to explore the mutations of spectrum and frequency in EC, 100 fresh-frozen tumors were analyzed. Figure 1 shows a summary of somatically altered genes that are recurrently mutated. Validated mutations were detected in 91 of the 100 tumors (91%), and 77 of the 100 tumors (77%) harbored concurrent mutations in two or more genes. Of 50 tumor-related genes, 37 were observed in EC tumors. Mutations were most frequently detected in *PTEN* (57%), *PIK3CA* (51%) and *TP53* (30%) (Fig. 1). *KRAS*, *CTNNB1*, *FBFR2*, *FBXW7* and *RB1* mutations were relatively frequent, with respectively 23%, 21%, 13%, 10% and 9%, followed by *APC* (6%), *SMARCB1* (5%), *AKT1*, *ATM*, *BRAF*, *ERBB2* and *MET*, (4%) *ERBB4*, *GNAS*, *KIT*, *SMAD4*, *SMO* and *STK11* (3%) *ABL*, *FGFR3*, *HNFB1A*, *KDR* and *NRAS* (2%) *EGFR*, *CDH1*, *CDKN2A*, *EZH2*, *EGFR1*, *GNAQ*, *IDH1*, *JAK2*, *MLH1*, *MPL*, *NOTCH1* and *VHL* (1%) (Fig. 1). A total of 284 (mean, 2.84) mutations were detected; 212 (74.6%) missense mutations, 39 (13.7%) nonsense mutations, 26 (9.2%) frameshift indels and seven (2.5%) non-frameshift indels (Fig. 1).

We examined the differences in mutation rates between EEC and NEEC using a chi-squared test. The mutation rate was significantly higher in the EEC (93.9%, 77/82) than in the NEEC (77.7%, 14/18) ($P = 0.03$). The association between mutation frequencies and clinicopathological features was investigated by the Mann-Whitney U Test. Age, histology and tumor grade were significantly correlated with mutation frequencies (Fig. 2a, b, c). On the other hand, there were no significant associations between stage, MI, VI, LNM, or recurrence and mutation frequencies (Supplementary Table 1).

Relationships Between Mutation Status And Clinicopathological Characteristics

Table 2 shows a summary of the associations between eight most frequently mutated genes (*PTEN*, *PIK3CA*, *TP53*, *KRAS*, *CTNNB1*, *FBFR2*, *FBXW7* and *RB*) and clinicopathological characteristics in EC patients. Patients with *PTEN* and *CTNNB1* mutations had a significantly younger age (< 60) than those without these mutations ($P = 0.003$ and $P = 0.006$, respectively). The *TP53* and *FBXW7* mutations were significantly observed in late-stage EC ($P = 0.017$ and $P = 0.023$, respectively), whereas *PTEN* mutation was significantly more common in early-stage EC ($P = 0.007$). *TP53* mutation was significantly more common in NEEC tumors ($P = 0.001$), whereas the *PTEN* and *CTNNB1* mutations were significantly more common in EEC tumors ($P = 0.001$ and $P = 0.02$, respectively). *TP53* mutations correlated with high-grade tumor ($P = 0.0001$). *FGFR2* mutations were significantly observed in deep ($\geq 1/2$) MI ($P = 0.016$). On the other hand, the association of *CTNNB1* mutation with superficial ($< 1/2$) MI was marginally significant ($P = 0.071$). *FBXW7* mutations significantly correlated with VI ($P = 0.001$). The association between *TP53* mutation and VI was also marginally significant ($P = 0.088$). In contrast, the association between *CTNNB1* mutation and non-VI was marginally significant ($P = 0.064$). *FBXW7* mutations significantly correlated with LNM ($P = 0.029$). The association of *TP53* mutation with LNM was also marginally significant ($P = 0.056$). On the other hand, the association of *PTEN* mutation with non-LNM was also marginally significant ($P = 0.08$). *TP53* mutation was significantly associated with recurrence ($P = 0.004$), whereas *PTEN* mutation was significantly associated with non-recurrence ($P = 0.001$).

Table 2
Frequency of
PTEN, *PIK3CA*,
TP53, *KRAS*,
CTNNB1, *FGFR2*,
FBXW7, and *RB*
mutations
according to
demographic and
clinicopathological
characteristics.

Correlations Of Mutation Status With Clinical Survival

The relationship between the eight most frequently mutated genes (*PTEN*, *PIK3CA*, *TP53*, *KRAS*, *CTNNB1*, *FBFR2*, *FBXW7* and *RB1*) and clinical survival was analyzed by log-rank test. The Kaplan-Meier curves for *PTEN*, *TP53* and *CTNNB1* mutations in EC patients are shown in Fig. 3. Patients with *PTEN* and/or *CTNNB1* mutations had a significantly better OS than those without these mutations ($P = 0.019$ and $P = 0.033$, respectively) (Fig. 3a, b), whereas those with *TP53* mutation had a significantly worse OS ($P = 0.001$) (Fig. 3c). However, *PIK3CA*, *KRAS*, *FBFR2*, *FBXW7* and *RB1* mutations had no significant associations with OS.

Discussion

Our findings showed a very high rate (91%) of a potential oncogenic driver or drug targetable mutations in EC patients. Validated mutations have been also reported in 78–94% of EC patients using a cancer panel [13, 14], and EC is known as a tumor with a high frequency of mutated genes. The comprehensive genomic analysis by The Cancer Genome Atlas (TCGA) resulted in the molecular classification of EC into four distinct subgroups [6]: the POLE group has an exceedingly high mutation rate in conjunction with somatic mutations in the exonuclease domain of *POLE*; The microsatellite instability (MSI) group has a high mutation rate and is a hallmark of a defective mismatch repair system because of hypermethylation of the *MLH1* promoter or a germline mutation in one of the mismatch repair genes; the copy number (CN)-low group has a low mutation rate and most of the microsatellite stable EEC; and the CN-high group has a low mutation rate and consists serous carcinoma with extensive high-level CN alterations and frequent somatic mutations in *TP53*. POLE, MSI and CN-low are almost exclusively of endometrioid morphology, whereas serous carcinoma contained in NEEC is almost exclusively in the CN-high group. POLE and MSI tumors have a high mutation frequency, which is approximately 100-fold and 6-fold greater than that seen in CN-low or CN-high tumors, respectively. POLE-mutated tumors are frequently of high-grade endometrioid histology. In the present study, the mutated frequencies were significantly higher in high-grade EEC tumors than in low-grade EEC tumors. Moreover, EEC tumors had a significantly higher mutation frequency than NEEC. Although we did not examine integrated *POLE* mutation, or perform MSI assay or CN analysis, our findings were consistent with those of TCGA.

We here investigated the associations between the eight most frequently mutated genes and prognostic risk factors or survival in EC patients. *PTEN* is identified as a tumor suppressor that is mutated in a large number of cancers at high frequency. *PTEN* was found to be mutated at the highest frequency in the present study. In particular, the rate of *PTEN* mutations in the EEC tumors was significantly higher than that in the NEEC tumors. The difference in the prevalence of *PTEN* mutation between EEC and NEEC was similar to that in previous studies [6, 15]. Since *PTEN* mutation is present in a relatively large proportion of atypical endometrial hyperplasia, which is known as a precancerous lesion, it may be considered an early event in the pathogenesis of EEC tumor [16, 17]. *PTEN* mutation is frequently observed in type I EC and is correlated with young age, early-stage, endometrioid subtype, low-grade and favorable prognosis as suggested by previous studies [18, 19], similar to our results.

PIK3CA is one of the most commonly mutated genes in solid cancers, and the majority of *PIK3CA* mutations cluster in hotspot regions in exon 9 (the helical domain) and exon 20 (the kinase domain) [20]. The *PIK3CA* mutation has been frequently detected in not only EEC but also NEEC [21, 22] as in the present study. As for survival, no significant association has been reported between *PIK3CA* mutations and disease-free survival in 94 EC patients [10]. On the other hand, associations have been reported between *PIK3CA* missense mutation and unfavorable outcome in grade 3 EEC tumors, and between *PIK3CA* exon 9 mutations and reduced survival in EC patients [23, 24]. In the current study, there were no significant associations between the *PIK3CA* mutation and any clinicopathological features or survival. A consensus on mechanism by which *PIK3CA* affects EC prognosis has yet to be achieved. Evaluation of the relationship between *PIK3CA* mutation and risk factors requires individualization with large samples.

Tumor suppressor p53 plays an important role in the preservation of genomic stability from various damages through the regulation of cell-cycle checkpoints, DNA repair, senescence and apoptosis, and *TP53* is one of the most frequent alterations in human cancer. The frequency of somatic mutation in *TP53* was 30% in our study. In particular, *TP53* mutations in EEC were significantly fewer than those in NEEC, and the proportion was similar to that of a previous study [25]. In addition, the associations of *TP53* mutation with late-stage, high grade, and poor OS were statistically significant. Furthermore, *TP53* mutations had marginally significant associations with LNM. The CN-high category represents serous-like EC, and mostly incorporates *TP53* mutation, indicating a poor prognosis [6]. These results suggest that somatic mutation in *TP53* can be the best predictive biomarker for poor prognosis in EC patients, and has the opposite characteristic of *PTEN* mutation.

The *KRAS* proto-oncogene regulates cell division as a result of its ability to relay external signals to the cell nucleus. Activating mutations in the *KRAS* gene promotes down-regulation of MAPK or PI3K/AKT, which further results in excessive cell proliferation and subsequent carcinogenesis. *KRAS* mutation is present in up to 25% of all human tumors, and this is one of the most frequently activated oncogenes [26]. *KRAS* mutations in EC have been mostly associated with type I estrogen-dependent EC, and their frequency is estimated around 10–30% [27–29]. Although *KRAS* mutation was also found in 24% (20/82) of the EEC tumors, there was no significant difference between EEC and NEEC in the present study. *KRAS* mutation is a relatively common event in endometrial carcinogenesis, but its prognostic value is limited.

Sideris et al reported that a consensus on the exact way that *KRAS* overall affects EC prognosis has yet to be achieved [30]. In the present study, no significant correlation between *KRAS* mutation and OS was observed, so the prognostic significance of *KRAS* mutation remains controversial.

CTNNB1 is a gene involved in the Wnt signaling pathway, which regulates cell growth, motility and differentiation. *CTNNB1* mutation is the activation of the Wnt signaling pathway, and has been specifically shown to be associated with carcinogenesis in many types of tumors [31]. Since *CTNNB1* mutations seemingly occur in atypical endometrial hyperplasia, *CTNNB1* mutation plays a critical role in the initiation and early progression of EEC tumors [32]. Other studies identified an increase in *CTNNB1* mutations in EEC compared to NEEC [33, 34], and no *CTNNB1* mutation was observed in the NEEC tumors in the present study. *CTNNB1* mutation was detected in 25.6% (21/82) of EEC tumors, which was a similar frequency to that previously reported in this series [33–35]. We here demonstrated that *CTNNB1* mutation was significantly associated with young age, endometrioid subtype and favorable OS, and borderline significantly associated with early-stage, superficial MI and VI. The clinicopathological features of *CTNNB1* mutations were similar to those of *PTEN* mutations; 90% (19/21) of tumors with *CTNNB1* mutation also had *PTEN* mutation. These data suggest that *CTNNB1* mutation may be a favorable prognostic biomarker in addition to *PTEN* mutation. However, recent studies have described significant associations between *CTNNB1* mutations and poor outcome among low-risk EEC patients, suggesting prognostic significance of *CTNNB1* mutation [35–38]; thus, the clinical significance of *CTNNB1* mutation among low-risk EEC tumors needs to be carefully interpreted.

FGFR2 is one of the receptors for fibroblast growth factor, and has been shown to be activated in a number of cancers through a variety of mechanisms, including gene amplification, translocations, and point mutations [39]. Some studies subsequently reported a *FGFR2* mutation frequency of 10–16% in EC tumors [34, 40, 41], and said frequency in the current study was 13%. In the present study, there was a significant association between *FGFR2* mutation and deep MI. To the best of our knowledge, these findings have not yet been reported. Jeske et al. suggested that clinical trials testing the efficacy of FGFR inhibitors in the adjuvant setting to prevent recurrence and death are warranted because *FGFR2* mutations are associated with poor outcomes in EEC [41]. Although we did not investigate the relationships between *FGFR2* mutations and OS, *FGFR2* mutations may contribute to a poor prognosis for EC with MI.

FBXW7 is a critical tumor suppressor that regulates proteasome-mediated degradation of various oncoproteins, such as cyclin E, c-Myc, Mcl-1, mTOR, Jun, Notch and AURKA in human cancer [42]. *FBXW7* mutations have been reported in several types of human cancers and found in 2.54% across all human tumors, according to COSMIC database meta-analyses [43]. Previous studies subsequently reported an *FBXW7* mutation frequency of 2–16% in EC tumors [28, 44], and said frequency in the current study was 10%. In particular, *FBXW7* mutation is observed in 20–30% of serous EC [6, 21, 22] and was also found in 30% (3/10) in the present study (data not shown). The results of the present study showed that *FBXW7* mutation correlated with late-stage, VI and LNM. In particular, the relationship between *FBXW7* mutation and VI in EC was a novel finding. Loss of *FBXW7* function caused by mutation resulted in high

Brg1 expression, and was consequently associated with VI, LNM and distant metastasis in gastric cancer [45]. Although there was no significant association between *FBXW7* mutation and OS in the current study, *FBXW7* mutation may be a predictive biomarker for poor prognosis.

The *RB1* gene, located on chromosome 13, is a well-known tumor suppressor gene that was discovered in genetic studies of hereditary retinoblastoma [46]. Defects in this gene are a cause of childhood cancer, bladder cancer, and osteogenic sarcoma. Loss of heterozygosity was reported in 18% of *RB* genes in EC and pRB downregulation was consistent with loss of heterozygosity [47]. Although *RB* mutation was detected in 9% of EC patients, no significant association was observed between clinicopathological features or survival and *RB* mutation. Our data indicate that inactivation of *RB1* was not a useful biomarker for EC.

However, the present study must be interpreted with caution, and a few limitations should be kept in mind. Said limitations include the small sample size, heterogeneity of histology or treatment within the cohort, and short follow-up period as they may have affected the association between somatic mutations and clinical outcomes. Another limitation is that our analysis relied on sequencing with a cancer hotspot panel, and only explained a portion of the total genetic alterations. The NGS platform used in this study detected only single nucleotide variants, and thus it was impossible to detect copy number variants and MSI status. Therefore, a large number of samples with long-term follow-up need to be prospectively analyzed using whole exome or genome sequencing that can detect single number variants, copy number variants and MSI status. Furthermore, other novel genetic or epigenetic alterations should be explored as well.

Conclusion

We demonstrated that our comprehensive NGS analysis using a cancer panel was feasible for mutational profiling of EC tumors. Although the mutated gene significance for clinical outcomes in EC patients was similar to that found in previous work, new findings, the correlations between *FGFR2* mutation and deep MI and between *FBXW7* mutation and VI, were revealed. Our current study suggests that EC patients can benefit from molecular profiling with predictable prognostic factors and select systemic adjuvant chemotherapy in combination with specific targeted therapies. We believe that this work is useful for understanding and evaluating the molecular characteristics of EC patients, and will lead to the establishment of novel treatment strategies that improve the survival of patients with EC in the future.

Abbreviations

EC

Endometrial carcinoma, OS:Overall survival, MI:Myometrial invasion, VI:Vascular invasion, LNM:lymph node metastasis, FIGO:International Federation of Obstetrics and Gynecology, NGS; Next-generation sequencing, EEC:Endometrioid endometrial carcinoma, NEEC:Non-endometrioid endometrial carcinoma, TCGA:The Cancer Genome Atlas

Declarations

Authors' contributions

TW, SW and KF conceived and designed the experiments. TW, MK, SN, SF, SS and KF collected samples and clinical data. HN, DT, TI, JI and SW performed molecular profile. TW analyzed the data and wrote the paper.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Fukushima Medical University School of Medicine (No. 1953), and informed consent was obtained from all patients. All participants signed informed consent forms.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

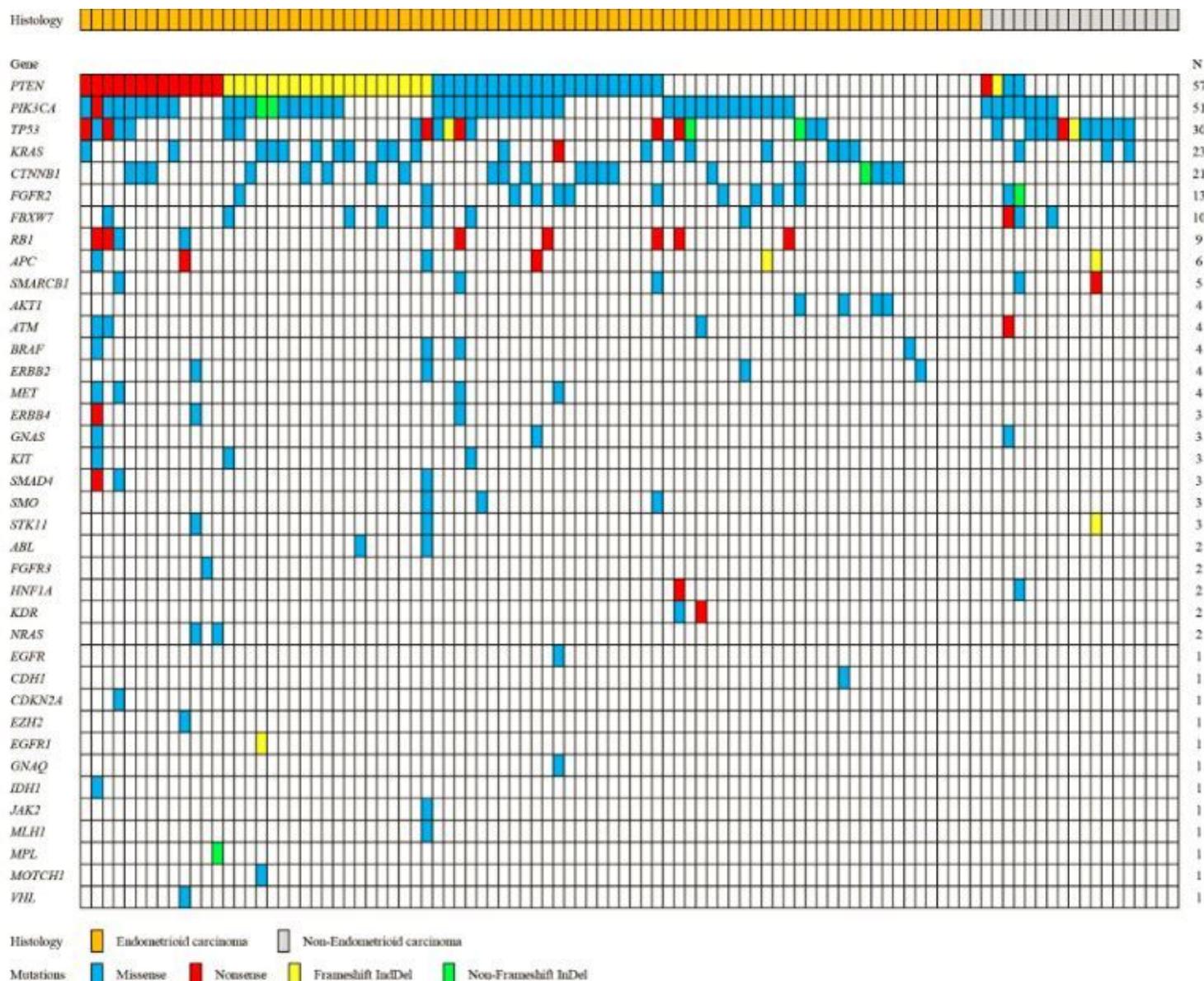


Figure 1

Summary of the relationships between somatic mutations and histological characteristics of endometrial cancer. All panels are aligned with vertical tracks representing 100 individuals.

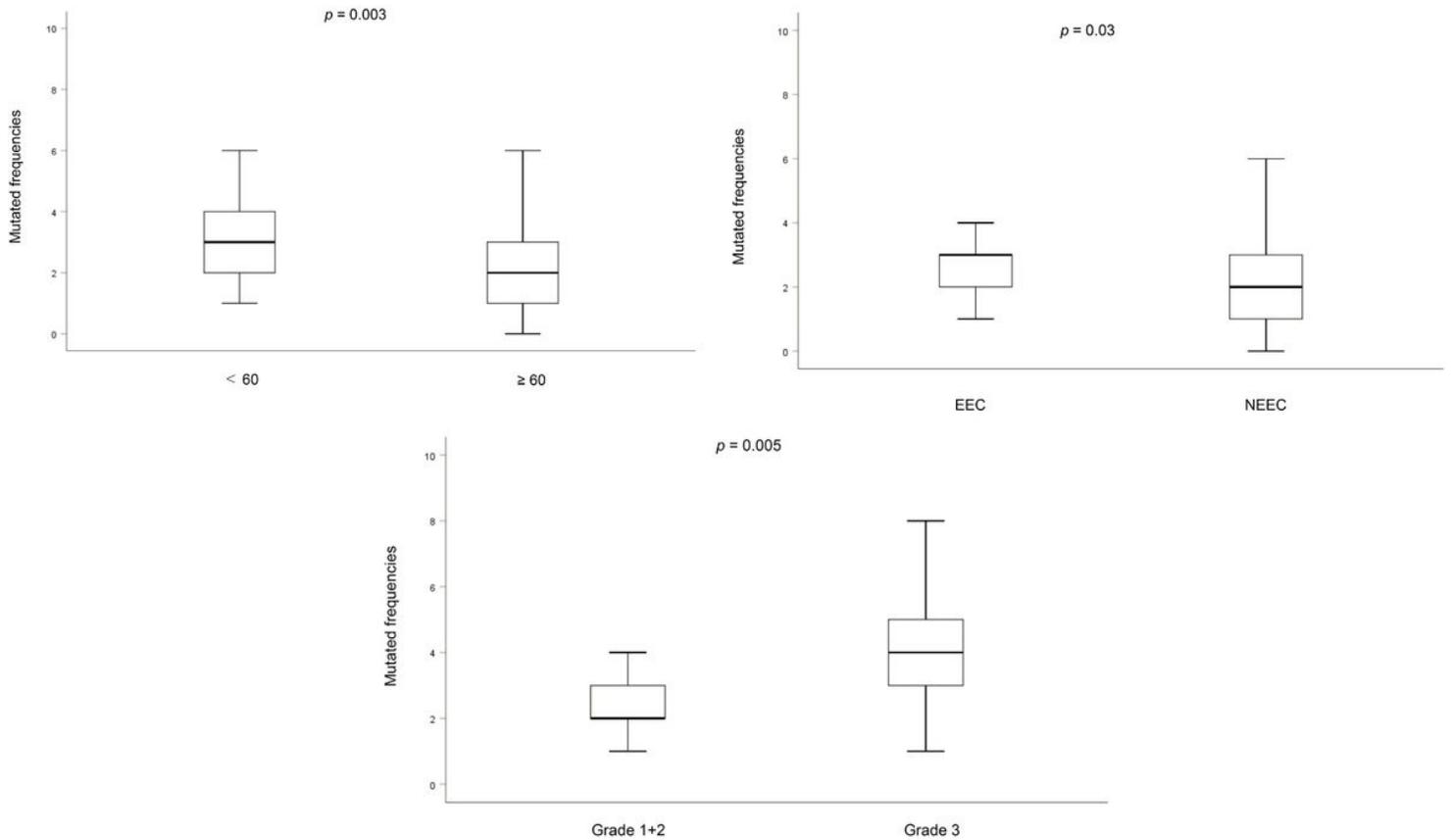


Figure 2

Box plot showing differences in mutation frequency between (a) < 60 and ≥ 60 ($P = 0.003$). (b) EEC and NEEC ($P=0.03$). (c) Grade 1 + 2 and Grade 3 ($P = 0.005$) EEC: endometrial endometrioid carcinoma, NEEC: non-endometrial endometrioid carcinoma

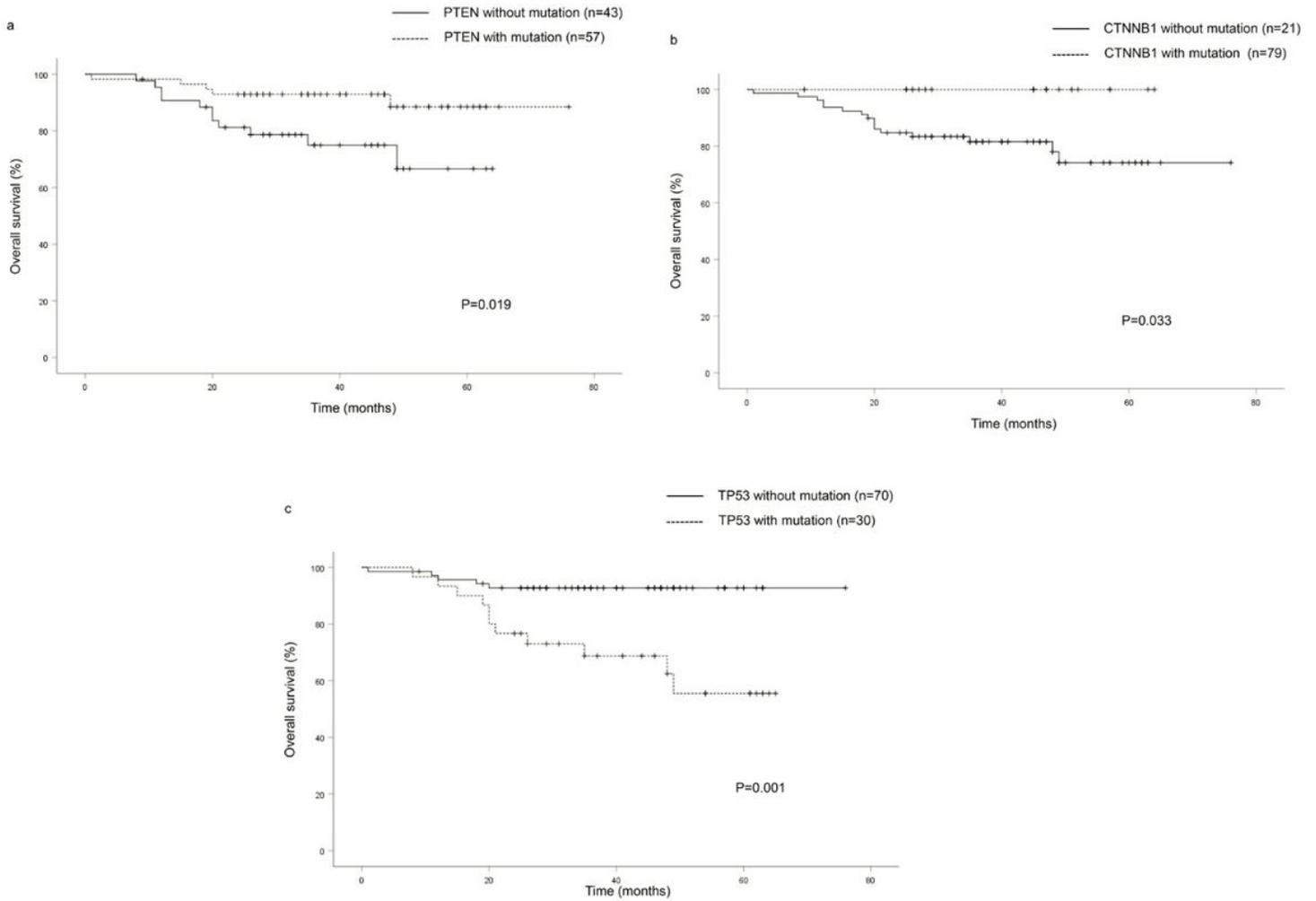


Figure 3

The Kaplan-Meier curves of overall survival in patients with endometrial cancer. (a) Patients with and without PTEN mutations (P = 0.019). (b) Patients with and without CTNNB1 mutations (P = 0.033). (c) Patients with and without TP53 mutations (P = 0.001).