OncoTargets and Therapy

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ORIGINAL RESEARCH

POLE Mutation Characteristics in a Chinese Cohort with Endometrial Carcinoma

This article was published in the following Dove Press journal:
OncoTargets and Therapy

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Objective: To study the characteristics of *polymerase epsilon* (*POLE*) exonuclease domain mutations in Chinese patients with endometrial carcinoma (EC).

Methods: This study analyzed data from 529 patients with EC in The Cancer Genome Atlas (TCGA) and 467 EC patients evaluated at the Shanghai First Maternity and Infant Hospital (SFMIH). *POLE* mutation heterogeneity was analyzed in paired curettage and hysterectomy samples from 120 SFMIH patients. Sanger sequencing identified mutations in the *POLE* exonuclease domain, and correlations between *POLE* mutation status and various clinicopathological features were determined by chi-squared testing and Cohen's kappa analysis, with Kaplan–Meier survival curves generated to assess correlations between *POLE* mutation status and overall survival (OS).

Results: Thirty-five mutations were identified in 467 samples (7.5%), and novel mutations were detected in the SFMIH cohort. Compared to the TCGA cohort, the SFMIH cohort had fewer *POLE* mutations when matched by age (<60) and histology (endometrioid) ($p < 0.001$ and $p = 0.010$, respectively). In our study cohort, *POLE* mutations were significantly associated with adjuvant treatment ($p = 0.029$), and patients with *POLE* mutations who underwent chemoradiotherapy had a poor OS ($p < 0.0001$). Notably, shorter OS was significantly associated with *POLE* mutations in hysterectomy samples from patients aged >60 years or with stage I disease in the paired curettage-hysterectomy group.

Conclusion: The significant difference in *POLE* mutation profiles between the TCGA and SFMIH cohorts, as well as the poor consistency between the curettage and hysterectomy samples, suggests that different parameters need to be applied to determine the prognosis of patients with EC in China.

Keywords: endometrial carcinoma, *polymerase epsilon* mutation, *POLE* mutation, Chinese cohort, hysterectomy, curettage specimens

Introduction

Endometrial carcinomas (ECs) are categorized into two subtypes according to their clinicopathologic characteristics. Approximately 70% to 80% of ECs are type I, with estrogen-dependent ECs often accompanied by a favorable prognosis. Type-II tumors account for ~10% to ~20% of ECs and exhibit more aggressive biological behaviors and poor outcomes.[1,2] Treatments are selected according to the histologic subtype and other clinicopathologic features associated with prognosis;[2–6] therefore, proper subtype classification is critical for selecting appropriate therapy. In 2013, The Cancer Genome Atlas (TCGA) project characterized ECs into four separate groups based on genomic features. A novel "ultramutated" subgroup harboring mutations in the exonuclease domain of the *polymerase epsilon* (*POLE*) gene was identified, often manifested as

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http://doi.org/10.2147/OTT.S258642

a microsatellite stable super-mutant phenotype with high somatic mutation rate. For example, Kandoth et al found a high mutant prevalence of *PTEN* (94%), *PIK3R1* (65%), *PIK3CA* (71%), *FBXW7 (82%)* and *KRAS* (53%) in 17 ultramutated samples.[7] Roberts et al detected 30 *POLE* mutations (5.6%) in 535 endometrioid carcinomas, which was lower than that in TCGA.[8] In the Church study, *POLE* mutations were detected in 48 out of 788 (6.1%) endometrial cancers. In FIGO grade 3 endometrioid carcinoma, the *POLE* mutation rate is high, ranging from 15%-22%.[7,9,10] Furthermore, Hussein et al observed that the endometrial carcinoma with *POLE* mutation was mainly inclined toward endometrioid differentiation, 60% of which were high-grade endometrioid carcinoma.[11] However, *POLE* mutations, mainly referring to *POLE* exonuclease domain mutations in the present study, may be associated with outcomes in which studies involving Chinese patients are lacking.

Multiple studies identified *POLE* mutations in several types of malignant tumors, especially in ECs, colorectal carcinomas, and gliomas.[9,12-14] *POLE* encodes DNA *polymerase epsilon*, which is responsible for chromosomal DNA replication during cell division.[15,16] Polymerase proofreading represents one of the primary mechanisms ensuring DNA replication fidelity.[17,18] Moreover, inactivation or suppression of the proofreading capacities of DNA polymerases can cause a dramatic increase in the number of spontaneous mutations.[11,19] In ECs, mutations in the exonuclease domain of *POLE* were previously identified primarily in hotspot regions, including exons 9, 13, and 14.[10,20] *POLE* mutations tend to occur in patients with FIGO grades 2–3, and *POLE* mutant FIGO grade 3 ECs are less prone to recurrence.[10] A study of 534 endometrioid carcinomas showed that 30 (5.6%) were *POLE* mutations, and the recurrence rate was approximately 3.4%, while the *POLE* wild type had a recurrence rate of 17%.[21] At the same time, Stenzinger et al found that the prognosis of colorectal cancer patients with *POLE* mutations was not significantly different from that of wild-type patients, but the mortality rate of patients with adjuvant or palliative chemotherapy was significantly higher than that of *POLE* wild type for terminal stage.[22]

In this study, we compared *POLE* mutation frequencies and clinicopathologic features between a TCGA database cohort, a cohort of predominantly non-Asian people, and a Chinese cohort of patients with EC and analyzed the effects of *POLE* mutations on overall survival (OS) in these two cohorts. Additionally, we determined the consistency of *POLE* mutation status between curettage specimens and hysterectomy specimens and examined the associations between mutation statuses and OS.

Materials and Methods

Patient Cohorts

In this study, we used the following two cohorts: our local cohort of patients with EC from Shanghai First Maternity and Infant Hospital (SFMIH) and the TCGA cohort. Specimens from the SFMIH cohort, including 467 primary tumor samples and 120 paired curettage specimens, were derived from EC patients treated at the department of gynecology (SFMIH) between 2007 and 2018. Clinical data were obtained by retrospective chart review, disease-specific death was defined as death due to EC and excluded death from other causes, and OS was defined as the time from diagnosis to death. Ethical approval was received from the institutional research ethics board. Current guidelines for adjuvant chemotherapy and radiotherapy (NCCN clinical practice guidelines (2018)[23]) rely on the International Federation of Gynecology and Obstetrics (FIGO) grade, FIGO stage, histotype, and lymphovascular-invasion (LVI) status. For the TCGA cohort, we downloaded the clinical and *POLE* mutation data from the EC interface of the TCGA portal (https://portal.gdc.cancer.gov/projects/TCGA-UCEC), resulting in data from 529 patients with ECs, 51 of whom harbored *POLE* mutations.

DNA Isolation

Before DNA extraction, samples are enriched by macrodissection to increase the content of tumor cells. DNA was isolated using a GeneRead DNA kit (Qiagen, Hilden, Germany) from tumor-rich regions (at least 50% tumor cells) of formalin-fixed, paraffin-embedded samples, as assessed by two independent pathologists. In samples with mutations, nontumor endometrium was also extracted from the same slide and underwent the same determination of somatic mutations.

Targeted Sequencing and Analysis

Mutations in the exonuclease domain of *POLE* (amino acids 268–471) were previously identified mainly in exons 9, 13, and 14.[10,20] The primer sets used covered these regions and are described later in this subsection. Polymerase chain reaction (PCR) amplifications were performed using 2×Taq master mix (Vazyme Biotech, Nanjing, China), and PCR products were purified using

Table 1 Clinical Features of Patients in the TCGA and SFMIH Cohorts

Characteristic	Subcategory	TCGA (n = 529)	SFMIH (n = 467)	Chi-Squared Test p
Age (y)	≥60 <60	352 (66.5%) 174 (32.9%)	142 (30.4%) 325 (69.6%)	<0.001
Clinical stage	I II III IV	330 (62.4%) 51 (9.6%) 121 (22.9%) 27 (5.1%)	388 (83.1%) 37 (7.9%) 38 (8.1%) 4 (0.9%)	<0.001[a]
Grade	1 2 3 Mixed	96 (18.1%) 116 (21.9%) 295 (55.8%) 22 (4.2%)	320 (68.5%) 58 (12.4%) 62 (13.3%) 13 (2.8%)	<0.001[a]
Histotype	Endometrioid Serous Mixed Clear Carcinosarcoma Undifferentiated	396 (74.9%) 111 (21.0%) 22 (4.2%) 0 (0.0%) 0 (0.0%) 0 (0.0%)	398 (85.2%) 34 (7.3%) 13 (2.8%) 7 (1.5%) 14 (3.0%) 1 (0.2%)	<0.001[a]

Note: [a]Bonferroni-corrected p-value.

a QIAquick gel extraction kit (Qiagen) according to the manufacturer's instructions, followed by Sanger sequencing. The primers used were as follows: *POLE*-Exon 9 forward, 5′-GTGTTCAGGGAGGCCTAATG-3′ and reverse, 5′-CCATCCCAGGAGCTTACTTC-3′; *POLE*-Exon 13 forward, 5′-CCTGGCTTCTGTTCTCATTCT-3′ and reverse 5′-GATGTGGCTCACATGCCT-3′; and *POLE*-Exon 14 forward 5′-GACCCTGGGCTCT TGATTT-3′ and reverse 5′-GGACATCCACCTCCAT TCAG-3′. Sequencing reactions were performed as previously described,[24] and all mutation-positive samples were resequenced to confirm the mutational status.

Statistical Analysis

Among the two study cohorts and the different tumor types, the chi-squared test or Fisher's exact test was used to assess significant differences in *POLE* exonuclease domain-mutation status. Kaplan–Meier curves and log-rank statistics were applied to determine differences in the univariate OS analyses. Cohen's kappa analysis was performed to determine the consistency of *POLE* mutation status between hysterectomy and curettage specimens. For all analyses, $p < 0.05$ was considered statistically significant, and all statistical tests were two sided. Statistical analyses were performed using SPSS (v.20.0; IBM Corp., Armonk, NY, USA).

Results

Characteristics of the EC-Patient Cohorts

We retrospectively analyzed 467 EC patients at SFMIH, the majority of whom (69.6%) were <60 years of age (32.9% of the TCGA cohort were <60 years of age). In total, 91% of the patients in the SFMIH cohort had early-stage disease (defined as patients with FIGO stage I or II disease), which was greater than this proportion in the TCGA cohort (72%). The SFMIH cohort included a lower proportion of patients with high-grade tumors than the TCGA cohort (55.8% TCGA vs 13.3% SFMIH), including grade 3 endometrioid, serous, mixed, and clear-cell carcinomas. Other notable differences between the SFMIH cohort and the TCGA patient population were found in the tumor histotypes, with endometrioid and serous carcinomas accounting for 85.2% and 7.3% of the patients in the SFMIH cohort compared with 74.9% and 21.0% in the TCGA cohort, respectively (Table 1). These findings suggested that the clinical characteristics of EC patients in the TCGA cohort differed from those in the SFMIH cohort.

Comparison of *POLE* Mutations Between the TCGA and SFMIH Cohorts

Despite commonalities in the frequent mutations found in the *POLE* exonuclease domain between both cohorts, we

found notable differences in clinical features and outcomes. Among the 467 patients with EC in the SFMIH cohort and the 529 patients in the TCGA cohort, we identified 35 and 55 mutations, respectively, in *POLE* exons 9, 13, and 14 (Figure 1A). Twenty-four novel mutations were identified in the *POLE* exonuclease domain (amino acids 268–471) in the SFMIH cohort, including missense mutations and a truncating mutation. Only three hotspot mutations within the *POLE* exonuclease domain were found in both cohorts, suggesting that the *POLE* mutations in the SFMIH cohort differed from those in the TCGA cohort (Figure 1B and Table 2).

Several studies, including TCGA, had shown that *POLE* mutations were associated with outcomes. Before assessing the effect of *POLE* mutations on overall survival, baseline variables were examined using multivariate analysis, the results of which showed no significant differences in the clinical characteristics within each group of the SFMIH cohort (Table 3). We performed a detailed stratified analysis to further explore the differences in *POLE* mutations between the two study cohorts. In the SFMIH cohort, younger patients (age <60) and those with the endometroid subtype of EC had significantly fewer *POLE* mutations than their counterparts in the TCGA cohort ($p < 0.001$ and $p = 0.01$, respectively) (Table 4). Because previous findings showed that *POLE* mutations were associated with a favorable prognosis, we compared the effects of *POLE* mutations on OS in both cohorts. The presence of *POLE* mutations in the TCGA cohort remained a significant favorable prognostic factor for OS ($p = 0.010$) in both the age <60 years subtype ($p < 0.0001$) and the endometrioid tumor subtype ($p < 0.0001$). In the SFMIH cohort, the OS analysis grouped according to age and histotype did not show significant differences (Figure 2A and B).

These results suggested that *POLE* mutations of EC patients from China are unique and might not be a favorable prognostic factor for OS. The low incidence might weaken the positive effect of *POLE* mutation on the prognosis of EC.

The Clinical Significance of *POLE* Mutations in EC in the SFMIH Cohort

We then determined associations between mutations in the *POLE* exonuclease domain and patient clinicopathologic characteristics in the SFMIH cohort (Table 3). We followed the NCCN clinical practice guidelines (2018) to determine the treatment regimen for patients.[23] After stratifying according to age group, we found no significant difference in *POLE* mutations between younger (<60 years) and older (≥60 years) subgroups. All *POLE*-mutated tumors were stage I, stage II, or stage III, with no *POLE* mutations identified in stage IV tumors. The *POLE* mutation rate according to the stage of EC was as follows: 6.44% in stage I, 13.51% in stage II, and 10.53% in stage III, with no significant difference among the groups ($p = 0.275$) (Table S1). Surprisingly, the frequency of *POLE* mutations was significantly higher in patients who received adjuvant chemoradiotherapy than in patients who did not, which was a novel characteristic of the SFMIH cohort (Table S1). We then investigated whether mutations in the *POLE* exonuclease domain correlated with differences in the OS of EC patients receiving adjuvant therapy. Among patients who underwent chemoradiotherapy, those harboring *POLE* mutations had a significantly worse prognosis than those without *POLE* mutations ($p < 0.05$; Figure 3).

Mutations in the *POLE* Exonuclease Domain in Hysterectomy and Curettage Specimens

We randomly selected 120 curettage specimens paired with hysterectomy specimens from EC patients from the SFMIH cohort for *POLE* mutation detection. Sixteen tumors (13.3%) were found to harbor mutations in the *POLE* exonuclease domain in the definitive hysterectomy specimens, whereas 23 curettage specimens (19.2%) harbored *POLE* mutations (Table 5). Comparison of the curettage samples with the hysterectomy samples revealed similarities with respect to histological subtype and grade, although identification of *POLE* mutations was inconsistent between the two groups (Table 5). We performed survival analysis using a univariate model according to *POLE* mutations in the subgroups of older patients, patients with stage I disease, patients with superficial myometrial invasion, and patients who underwent chemotherapy. Interestingly, patients in the older subgroup, as well as the subgroup with stage I disease and *POLE* mutations in the hysterectomy specimens but not in the curettage specimens, had significantly worse OS than patients in the same subgroup without *POLE* mutations (Figure 4A and B). Furthermore, OS was not associated with *POLE*

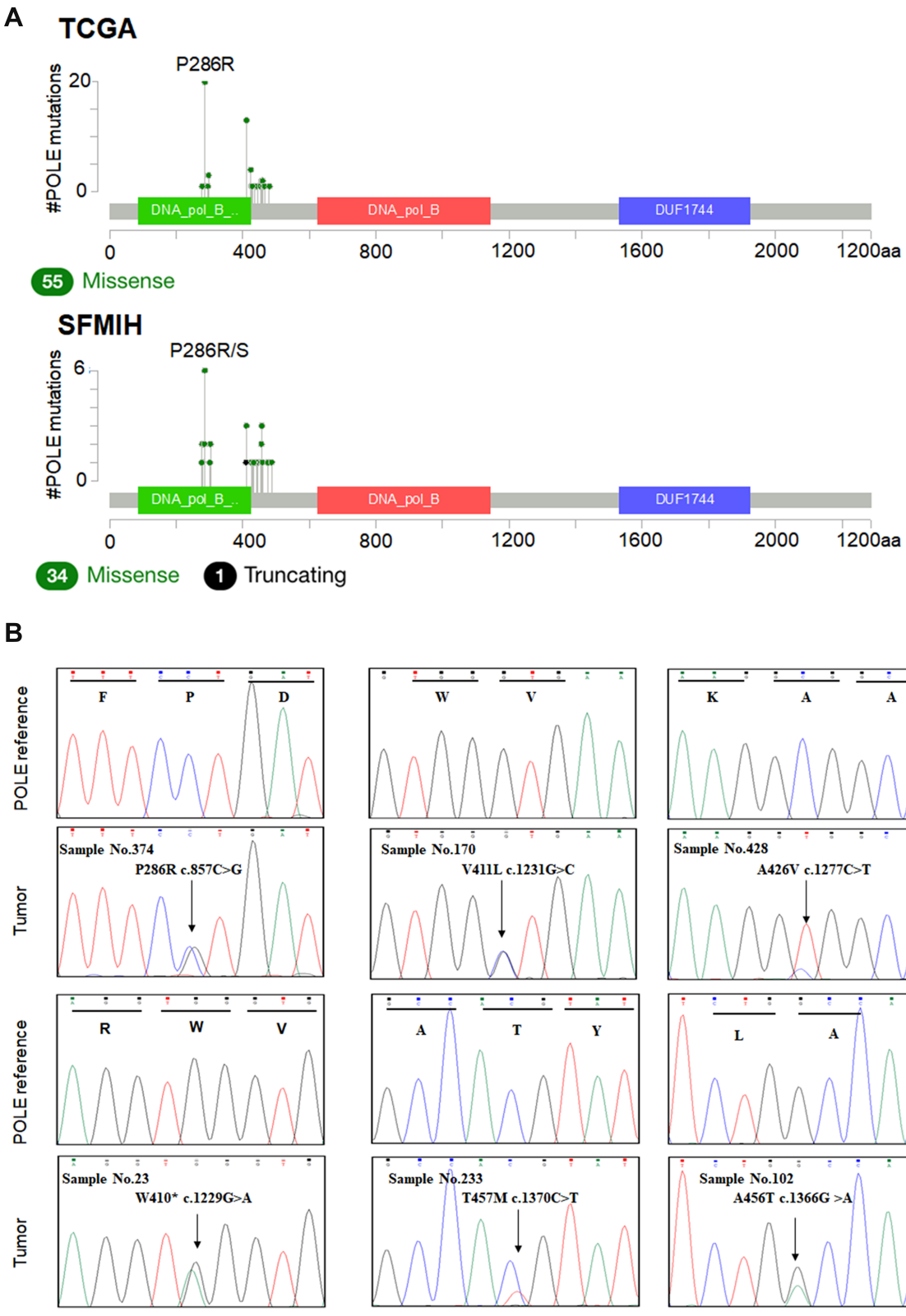

Figure 1 Mutations in the *POLE* exonuclease domain in ECs. (**A**) Mutations in the *POLE* exonuclease domain in the TCGA and SFMIH cohorts. (**B**) Hotspot *POLE* mutations in the SFMIH cohort.

Table 2 Sites of Mutations in the *POLE* Exonuclease Domain in ECs

	TCGA		SFMIH		
Histology	*POLE*-mutated	*POLE* mutation (n)	*POLE*-mutated	*POLE* mutation (n)	Common mutation
Endometrioid	51/396	**A456P, L424V, M444K**, P286R, V411L (3) **A465V**, P286R (7), **Q453R, S459F** A426V, **A456P, K429N, L424I** (3), **M295R**, P286R (11), **P436R, S297F** (3), **S461L, T278M**, V411L (9)	28/398	V411L (1), T278S (1), T457M (2), V411M (1), W410* (1), T278P (1), S459Y (1), P441L (1), P286R (3), L455Q (1), K429R (1), G433D (1), F285S (1), D301N (1), A456T (2), E277V (1), F285L (1), P286S (1), R446W (1), P476L (1), Y434N/V411L (1) P286R (1), Q303R (1)	*P286R V411L A426V*
Serous	3/111	**A480D**, V411L, **A428T**	4/34	A426V (1), M487V (1), T279A (1), T457L (1)	
Clear cell	0		1/7	P286R (1)	
Undifferentiated	0		0/1		
Mixed Carcinomas	1/22	P286R	1/13	Q303R (1)	
Carcinosarcoma	0		1/14	H475R (1)	
Total	55		35		

Notes: Text in bold represents *POLE* mutations specific to the TCGA cohort, text with underline represents *POLE* mutations specific to the SFMIH cohort, and text in italics represents *POLE* mutations common to both the TCGA and SFMIH cohorts.

Table 3 Patient Characteristics at Baseline in the SFMIH Cohort

	SFMIH (n = 467)	Mutated (n = 34)	Not Mutated (n = 433)	Chi-Squared Test
Age	median	54.5	56	ns
	IQR	51–59.5	49–61	
Grade				ns
	1	19 (55.88)	302 (69.75)	
	2	6 (17.65)	52 (12.01)	
	3	7 (20.59)	56 (12.93)	
	Mixed	2 (5.88)	11 (2.54)	
Clinical stage				ns
	I	25 (73.53)	363 (83.83)	
	II	5 (14.71)	32 (7.39)	
	III	4 (11.76)	34 (7.85)	
	IV	0 (0.00)	4 (0.92)	
Histology				ns
	Endometrioid	27 (79.41)	371 (85.68)	
	Serous	4 (11.76)	30 (6.93)	
	Mixed	1 (2.94)	6 (1.39)	
	Clear	0 (0.00)	1 (0.23)	
	Carcinosarcoma	1 (2.94)	12 (2.77)	
	Undifferentiated	1 (2.94)	13 (3.00)	

mutations in either the curettage or hysterectomy specimens of patients with myometrial invasion and a history of chemotherapy (Figure 4C and D).

Discussion

In this study, we identified somatic mutations in the *POLE* exonuclease domain in 7.28% of a large independent cohort of 467 patients with EC from SFMIH, China. We identified characteristics distinguishing the SFMIH cohort from the TCGA cohort, including age, stage, grade, and histotype, that might be related to differences in race between the two cohorts.[25] In the TCGA cohort, 55 *POLE* mutations were located in exons 9, 13, and 14, whereas 35 were detected in the SFMIH cohort. Furthermore, most hotspot mutations found in the TCGA cohort were not detected in our cohort, but we detected 24 novel somatic *POLE* mutations in the SFMIH cohort. Additionally, we explored differences in *POLE* mutations between subgroups of the TCGA and SFMIH cohorts, with the results confirming that in younger (<60 years) and endometrioid-histotype subgroups, the SFMIH cohort had fewer *POLE* mutations than the TCGA cohort. Moreover, previous studies investigating a TCGA cohort of patients with ECs showed that *POLE* mutations occurred predominantly in endometrioid tumors. In the SFMIH cohort, *POLE* mutations were found at higher frequencies in serous tumors than in endometrioid tumors, which was unexpected.

Survival analysis showed that no significant prognostic impact of *POLE* mutations on OS was identified within the young and endometrioid subgroups of the SFMIH cohort,

Table 4 Demographic and Clinical Characteristics of Mutations in the *POLE* Exonuclease Domain in the TCGA and SFMIH Cohorts

Indicators	Group	TCGA			SFMIH			Chi-Squared Test
		Wild-Type	*POLE* Mutations	*POLE* Mutations (%)	Wild-Type	*POLE* Mutations	*POLE* Mutations (%)	p
Age (y)	≥60	336	16	4.55	133	9	6.34	0.411
	<60	140	34	19.54	300	25	7.69	<0.001
Clinical stage	I	299	31	9.39	363	25	6.44	0.142
	II	46	5	9.80	32	5	13.51	0.841[a]
	III	107	14	11.57	34	4	10.53	1.000[a]
	IV	25	2	7.41	4	0	0.00	1.000[b]
Grade	1	89	7	7.29	302	19	5.94	0.631
	2	106	10	8.62	52	6	10.34	0.711
	3	261	34	11.53	56	7	11.29	0.958
	Mixed	21	1	4.55	11	2	15.38	0.268
Histology	Endometrioid	348	48	12.12	371	27	6.78	0.010
	Serous	108	3	2.7	30	4	11.76	0.089[a]
	Clear cell	0	0	0	6	1	14.29	——
	Undifferentiated	0	0	0	1	0	0	——
	Mixed carcinomas	21	1	4.55	12	1	7.69	0.698
	Carcinosarcoma	0	0	0	13	1	7.14	——
Total cases		**477**	**52**	**9.83**	**433**	**34**	**7.28**	**0.153**

Notes: [a]Corrected p-value. [b]Fisher's exact test.

which differed from previous reports, which presented better outcomes for patients with *POLE* mutations.[26–28] The higher frequency of low-risk (Grades 1 and 2) patients in the Chinese cohort (typically with good clinical outcomes), together with the pathogenicity of *POLE* EDM, could account for the discrepancies with previous literature regarding survival of patients with *POLE* mutated EC.

Interestingly, we found that among patients who received adjuvant chemoradiotherapy according to the treatment guidelines, those with *POLE* mutations had a significantly higher mortality rate than those without *POLE* mutations. This result is consistent with an in vitro study that showed that *POLE* mutant tumor cells have a significantly higher resistance rate to platinum drugs than the *POLE* wild-type cell line ($p < 0.004$),[29] indicating that *POLE* mutations contribute to the insensitivity to traditional chemotherapy.[30] These data support the possibility that *POLE*-mutated tumors, due to their defective polymerase proofreading capability, continue to acquire mutations during cell division; with the high tumor mutation burden, large amounts of tumor neoantigens are produced and may lead to insensitivity to adjuvant therapy. Studies have shown that *POLE* mutations do not play a significantly positive role in adjuvant treatment, and our results revealed a worse OS with adjuvant therapy.[31] In summary, further studies should investigate whether *POLE* mutations have prognostic or predictive implications in these patients and, if so, the nature of the underlying biological mechanism. In the present study, we did not find an association between *POLE* mutation status and progression-free survival (PFS) due to the relatively small number of patients who experienced recurrence; therefore, a longer follow-up time will be needed to address the significance of the prognostic impact of *POLE* mutations on PFS.

One novel objective of this study was to determine whether curettage and hysterectomy specimens were consistent in terms of the identified somatic mutations in the *POLE* exonuclease domain and whether such mutations in the curettage specimens could be used for predictions of

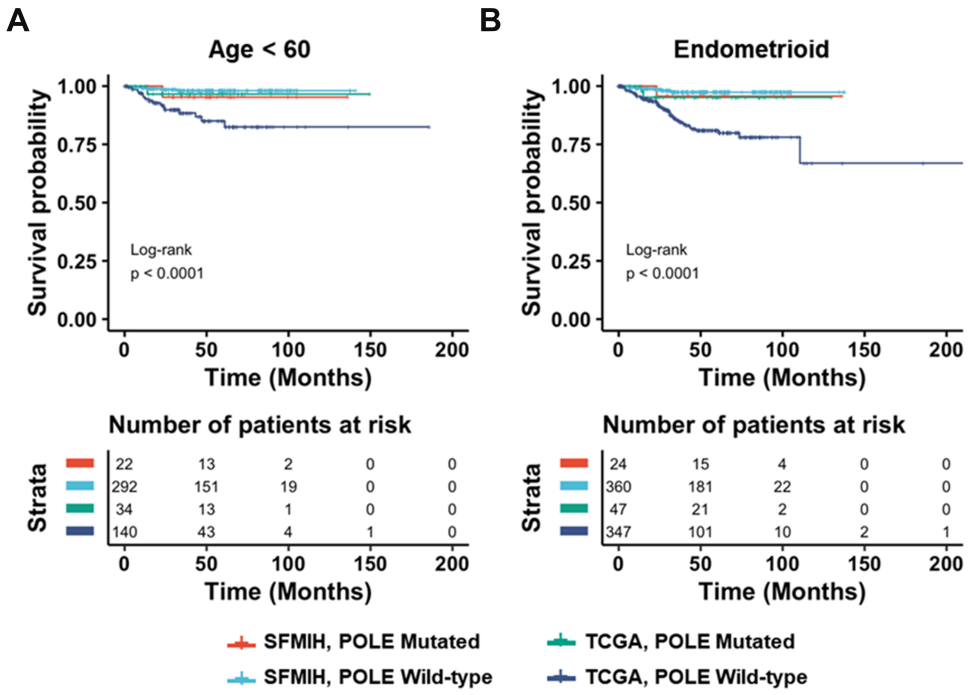

Figure 2 Kaplan–Meier estimates according to *POLE* mutation status in the TCGA and SFMIH cohorts. (**A**) OS for the younger cohort (<60 years) and (**B**) the endometrioid cohort. P-values were calculated using the Log rank test (two-sided).

prognosis. Various studies showed inconsistencies in tumor typing and histological grading between preoperative and hysterectomy specimens.[32,33] The present study showed good consistency for both pathological grade and histological type in paired samples from 120 patients with ECs. Unexpectedly, we identified more *POLE* mutations in curettage specimens than in hysterectomy specimens by Sanger sequencing, although a recent report showed that

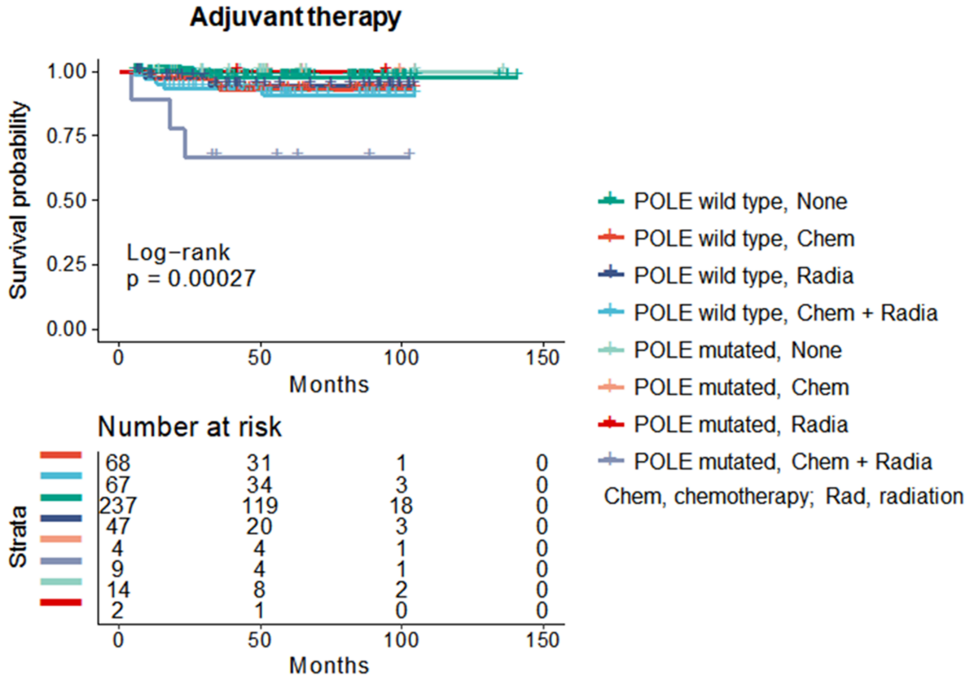

Figure 3 Among patients who underwent chemoradiotherapy, those harboring *POLE* mutations had a significantly worse prognosis than those without *POLE* mutations.

Table 5 Concordance of Tumor Features in Hysterectomy and Preoperative Curettage Specimens from the SFMIH Cohort

Characteristic	Subcategory	Curettage	Hysterectomy	Concordant	Disconcordant	Cohen's Kappa	
						κ-value	p
POLE	Mutated Not mutated	23 97	16 104	5 86	11 18	0.118	
Grade	1 2 3	78 16 17	77 21 21	68 12 16	9 8 6	0.641	<0.001
Histology	Endometrioid Serous Clear Sarcoma Atypical hyperplasia	98 10 3 1 8	104 12 3 1 0	95 10 2 1 0	9 2 1 0 0	0.647	<0.001

using DNA-based techniques to evaluate molecular alterations had high concordance rates between preoperative specimens and definitive hysterectomy specimens.[34,35] To determine whether the *POLE* mutations in curettage specimens differed from those in hysterectomy specimens, we grouped patients according to clinical characteristics for

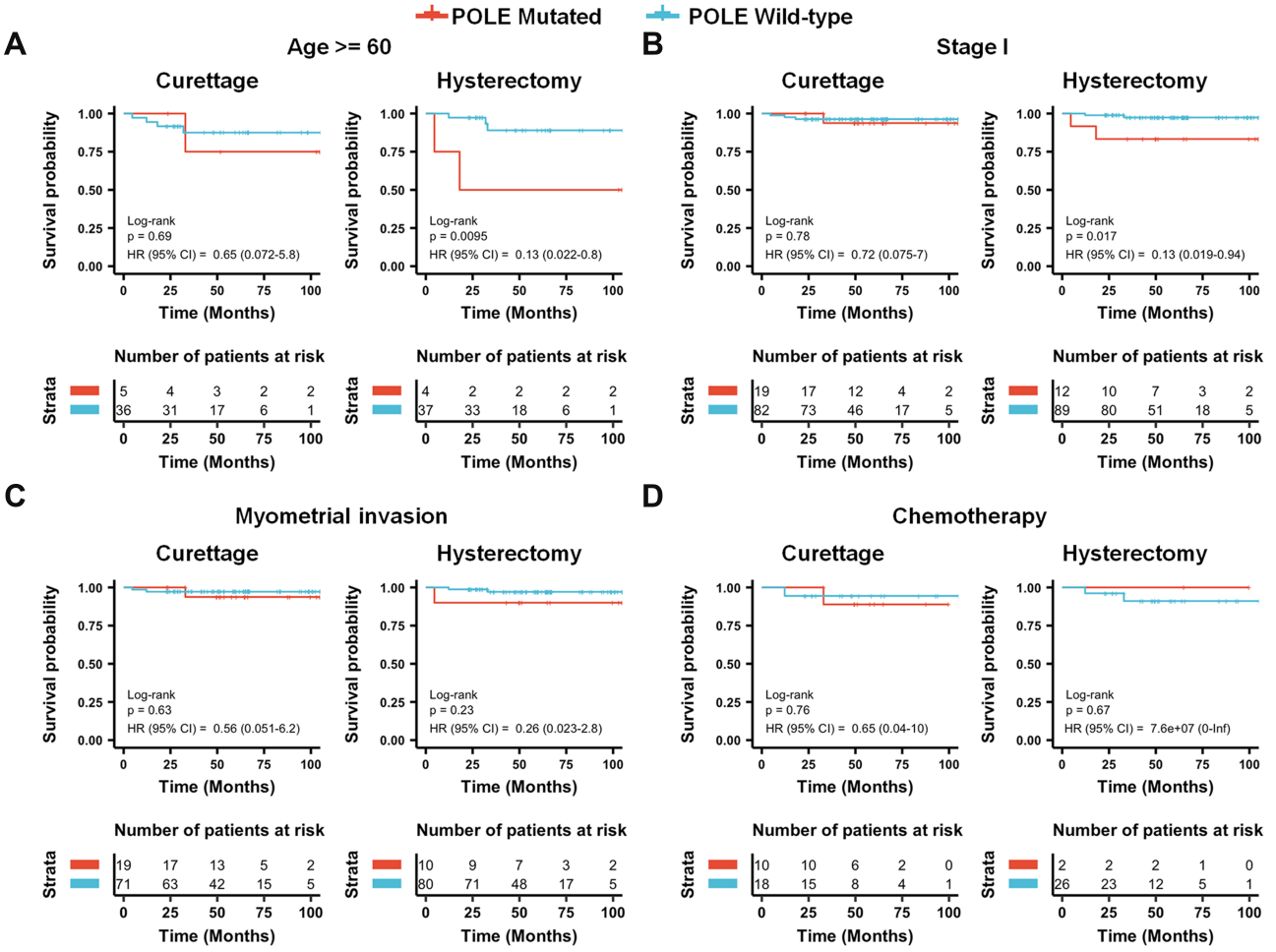

Figure 4 Kaplan–Meier estimates according to *POLE* mutation status in curettage and hysterectomy specimens. (**A**) OS for the older cohort (≥60 years), (**B**) the stage I cohort, (**C**) the myometrial-invasion cohort, and (**D**) the cohort having previously received chemotherapy. P-values were calculated using the Log rank test (two-sided).
Abbreviations: CI, confidence interval; HR, hazard ratio.

detailed stratified analyses. Our data indicated that *POLE* mutations result in preoperative tumors not accurately predicting the final *POLE* mutation results, especially in patients aged >60 or in those with stage I tumor, exhibiting superficial myometrial invasion, or having received adjuvant chemotherapy. Although this result is somewhat puzzling and counterintuitive, expression inconsistencies between curettage and hysterectomy specimens were not uncommon. For example, β-catenin staining was observed in the curettage specimens but not in the hysterectomy specimens[36] from patients with sporadic ECs. The reason behind this discordance needs to be further clarified. Recently, many researchers have tried to utilize molecular biomarkers in curettage specimens to predict the behavior of EC cells. However, the high inconsistency rate shown here indicates that this approach should be used very cautiously.

Furthermore, we also observed that identification of *POLE* mutation status could facilitate accurate patient counseling and help determine further treatment, thereby enabling tailoring of the extent of surgery and/or adjuvant therapies to the individual patient profile. Based on these findings, we explored whether *POLE* mutations in curettage or hysterectomy specimens correlated with OS. Importantly, we found that *POLE* mutations in hysterectomy specimens but not in curettage specimens were a significant predictor of poor OS among older patients and those with stage I disease. These findings suggested that identifying *POLE* mutations in curettage specimens was not helpful for determining the extent of treatment. However, this is the first study investigating the correlation of *POLE* mutations with prognosis in curettage specimens from subgroups of patients with different clinical features, and more studies are needed to determine their clinical significance.

Clinical Perspectives

The Cancer Genome Atlas (TCGA) project characterized endometrial cancer harboring *polymerase epsilon* (*POLE*) gene mutations as a "ultramutated" subgroup, with favorable outcomes. In the present study, total of 467 patients with EC were screened for the presence of mutations in the *POLE* exonuclease domain within exons 9, 13, and 14 in China. In contrast to the data obtained for the TCGA cohort, in which most patients were non-Asian, we found that *POLE* mutations might not be prognostic factors of poor OS for endometrioid patients with ECs in China. Moreover, we found that, for EC patients requiring chemotherapy combined with radiotherapy in China, *POLE* mutations might lead to a worse prognosis. This study represents the first analysis of correlations between *POLE* mutations in hysterectomy specimens with EC prognosis, but our results require further validation to clarify the role of *POLE* mutations in ECs in China.

Consent for Publication

The authors confirm that we have obtained written consent from the patients to publish this manuscript.

Abbreviations

POLE, *polymerase epsilon*; EC, endometrial carcinoma; TCGA, The Cancer Genome Atlas; SFMIH, Shanghai First Maternity and Infant Hospital; OS, overall survival.

Data Sharing Statement

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

Ethics Approval and Consent to Participate

We confirm that this study was conducted in accordance with the Declaration of Helsinki. The written informed consent for the biological studies was obtained from each patient involved in the study, and the study was approved by the Ethics Committee of The First Maternity and Infant Hospital of Tongji University School of Medicine. Reference number: KS18108; Date of approval: May 4, 2018.

Acknowledgments

We thank all patients involved in this study.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Funding

The study was supported by the National Natural Science Foundation of China (grant numbers 81472427, 81672574, and 81702547), the Shanghai Health System Outstanding Talents Program (grant number 2018YQ23), the Shanghai Sailing Program (grant number 17YF1415300), the Shanghai New Frontier Technology Project (grant number

SHDC12015110), and the Shanghai Municipal Medical and Health Discipline Construction Projects (grant number 2017ZZ02015).

Disclosure

The authors declare no potential conflicts of interest.

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