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Mutation spectrum of *POLE* and *POLD1* mutations in South East Asian women presenting with grade 3 endometrioid endometrial carcinomas

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HIGHLIGHTS

- Somatic *POLE* mutations are associated with improved recurrence free survival.
- Germline mutations in *POLE* and *POLD1* are seen in 8.5% of patients with grade 3 endometrioid endometrial carcinoma.
- Somatic- *POLE* mutated grade 3 endometroid endometrial cancers were more likely to be microsatellite stable and associated with peritumoral lymphocytic inflitrates.

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ABSTRACT

Objective. Somatic *POLE* mutations have been found in a subset of endometrioid ECs particularly in FIGO grade 3 tumors while *POLD1* mutations are reportedly rare in ECs. While it has been suggested that *POLE* mutation confers good prognosis, the data remains conflicting. Our study aims to determine the mutation spectrum of somatic and germline *POLE* and *POLD1* gene mutations in South East Asian (SEA) women with FIGO grade 3 endometrioid ECs.

Methods. Forty-seven patients diagnosed with FIGO grade 3 endometrioid EC, diagnosed between 2009 and 2013 were included. Next generation sequencing (NGS) using formalin fixed embedded (FFPE) tissue was utilized to sequence tumor and matched normal tissue. Tumors were also assessed for other clinicopathologic and microsatellite status phenotype. Survival curves for pathogenic somatic *POLE* mutated and wild-type tumors were estimated by Kaplan–Meier method.

Results. Pathogenic *POLE* (somatic or germline) and *POLD1* (germline) mutations were detected in 29.7% (14/47) and 4.3% (2/47) patients, respectively. Three pathogenic germline mutations; one *POLE* and two *POLD1* mutations were novel. Pathogenic germline and somatic *POLE* and *POLD1* mutations were associated with 100% recurrence free survival. In contrast, among the wild-type *POLE* and *POLD1* patients, 25% (8/32) had recurrence with 15.6% (5/32) subsequently dying of the disease. Somatic *POLE*-mutated tumors were more commonly associated with microsatellite stable (MSS) ECs (83% vs 49%; $p = 0.04$) and peritumoral lymphocytic infiltration (75% vs 42%; $p = 0.05$). All tumors with tumoral infiltrating lymphocytes exhibited peritumoral lymphocytic infiltrate but not vice versa.

Conclusion. Mutations in *POLE* and *POLD1* in SEA women with grade 3 endometrioid ECs are associated with improved recurrence free survival. Notably, germline mutations in either *POLE*/*POLD1* were seen in 8.5% of patients who will require appropriate genetic counseling regarding risk of developing colorectal carcinoma and on the need for additional surveillance for colonic changes. MSS and peritumoral lymphocytic infiltration may be useful histological features for distinguishing *POLE* mutated grade 3 endometrioid ECs.

1. Introduction

Endometrial carcinoma (EC) of endometrioid histotype is the most common gynecologic carcinoma among women in the developed

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world [1,2] with FIGO grade 3 endometrioid carcinomas associated with mortality rates similar to uterine serous carcinoma and clear cell carcinoma [3]. In 2013, the Tumor Cancer Genomic Atlas (TCGA) Research Network characterized endometrial carcinomas (ECs) into four separate groups according to their genomic profile; *POLE* ultramutated, microsatellite instability hypermutated, copy-number low, and copy-number high [4]. The *POLE* ultramutated tumors are characterized by mutations in the exonuclease domain of the *POLE* gene, mostly associated with FIGO grade 3 endometrioid ECs and a 100% progression-free survival rate [4,5]. The mean follow-up time censored to recurrence for all grade 3 endometrioid EC patients was 41 months in the TGCA study and 51 months in a subsequent study [4,5]. Another study failed to find an association between *POLE* gene mutation and superior progression-free survival rates [6]. Approximately 7% of ECs harbor pathogenic somatic *POLE* gene mutations [4,7] and when selected for FIGO grade 3 endometrioid ECs only, the mutation rate is 15% [5].

Aside from *POLE*, interest also extends to another polymerase associated gene, *POLD1* [8]. Both encode for polymerase proteins [9]. Currently, there is no known prognostic significance associated with *POLD1* mutation. Instead, attention is primarily centered on *POLD1* germline mutations and potential risk of developing secondary tumor in a hereditary syndromic manner [10]. Pathogenic mutations in either genes are also seen in colorectal carcinomas and are now considered part of the polymerase proofreading-associated polyposis syndrome, a dominantly inherited, highly penetrant syndrome [11]. These patients have oligoadenomatous polyposis and develop colorectal and endometrial carcinomas at a younger age. *POLE* mutations gene are more common in ECs patients while *POLD1* is more frequently found in colorectal adenocarcinoma patients [8,12]. *POLE* gene mutation is more commonly somatic while *POLD1* mutations in colorectal carcinomas and endometrial carcinomas are usually germline [8,12]. *POLD1* mutation hotspots in colorectal tumors are scattered throughout the many exons the gene [12] while published pathogenic mutations in ECs have been concentrated to exons 9 to 14 [4,6–8].

Histologically, *POLE* mutated tumors have been reported to exhibit areas with ambiguous features, easily mistaken for uterine serous carcinoma [13]. To date, suggestive histological features associated with *POLD1* mutated endometrial tumors have not been described.

Another phenotype of interest in both *POLE* and *POLD1* tumors is the microsatellite status. Although TCGA initially reported *POLE* mutated endometrial carcinomas to be microsatellite stable, this was later refuted when between 5.2% and 18% of *POLE* mutated tumors were shown to be microsatellite unstable-high (MSI-H) [4,6,13]. *POLD1* mutated colorectal tumors are far more likely to be microsatellite unstable [11,12]. This has led some to propose incorporation of *POLE* gene testing as part of the Lynch Syndrome screening [6,14]. Microsatellite status of *POLE* and *POLD1* mutations in ECs of Asian women has not been studied.

We examined the potential utility and precision of high throughput next generation sequencing in detecting somatic and germline *POLE* and *POLD1* gene mutations using only formalin fixed paraffin embedded (FFPE) tissue with FIGO grade 3 endometrioid ECs. With a gene target coverage beyond the current published exons 9–14 of the *POLE* gene, we aimed to discover new mutations associated with good prognosis. We concurrently sequenced the *POLD1* gene for pathogenic mutations to examine for tumors with microsatellite unstable phenotype.

2. Methods

2.1. Patient selection

A total of 47 women with FIGO grade 3 endometrioid ECs diagnosed and surgically staged between 1 January 2009 and 31 December 2012 at KK Women's and Children's Hospital (KKH) identified from the Singhealth Tissue Repository database. Only women who were managed and followed up at KKH were included. Tumor and matched normal tissue were obtained from FFPE tissue of the total hysterectomy specimen performed for tumor staging. Ethics approval was obtained from the Singhealth Centralised Institutional Review Board (CIRB Ref: 2014/792/B). Clinical data was obtained from retrospective inspection of clinical notes.

2.2. Histopathological assessment and clinical data

All the hematoxylin and eosin slides and any corresponding immunohistochemical markers requested as the initial time of diagnosis of each case were re-assessed by at least one specialist gynecologic pathologist (A.W. or S.M.). The histological parameters documented for each case were: tumor location in uterine corpus, the presence or absence of tumor infiltrating lymphocytes (TILs) define as ≥42 per high power field, and lymphovascular invasion. Lymph node status was also assessed as part of staging. All tumors were restaged to the 2009 FIGO staging system, where required. The clinical parameters recorded were; patients' age at diagnosis, treatment history, survival and recurrence data with disease specific death (DSD) defined as death from disease excluding death from other causes and recurrence-free survival (RFS) defined as time to clinical/radiological evidence of disease recurrence from the initial diagnosis. These patients were followed up to their death or to the most recent visit to 31 May 2015.

2.3. DNA extraction

DNA for both tumor and match normal tissue were extracted from FFPE samples using ReliaPrep gDNA FFPE kit, Promega. Slides were assessed by light microscopy for tumor-rich areas (>50 tumor cells) marked out by the pathologist (A.W.) for purification of tumor DNA. Matched normal DNA was obtained from the ovaries and/or fallopian tubes. Cases with metastatic carcinoma involving the ovary and/or fallopian tubes, matched normal DNA was purified from the uninvolved normal cervix or myometrium. Purified DNA was quantified using the Qubit (Invitrogen, Life Technologies).

2.4. Microsatellite instability

We used the Promega MSI Analysis System Version 1.2 which consists of five monomorphic mononucleotide markers (BAT-25, BAT-26, NR-21, NR-24, and MONO-27) to assess the MSI status of the ECs and two polymorphic pentanucleotide markers (Penta Cand Penta D) for sample identification. MSI analysis was performed according to the manufacturer's directions (Promega Corp.). Products were analyzed by capillary electrophoresis using an ABI 3100 Genetic Analyzer (Applied Biosystems). A tumor sample was classified as MSI-high (MSI-H) if two or more markers showed instability, MS-stable (MSS) if no instability was noted, and MSI-low (MSI-L) if a single marker revealed shift in loci peaks.

2.5. Ion Torrent PGM next-generation sequencing and analysis

A custom designed panel for *POLE* gene mutation testing using FFPE tissue was manufactured using the Ion AmpliSeq™ software. Tumor and matched DNA were sequenced for *POLD1* and *POLE* genes (see Supplementary Table 1) according to the manufacturer's protocol using 10 ng DNA, Ion AmpliSeq primer pool and Ion AmpliSeq Library Kit 2.0 Beta (Life Technologies). The Ion AmpliSeq™ custom design panel provided 95.7% (7035 out of 7351 base pairs) coverage of exonuclease regions of the *POLE* gene with 100% target coverage of the published hotspot exons of 9 to 14. Only exon 1 had no target coverage while 8 other exons contained missed target coverage (see Supplementary Table 1). The AmpliSeq design for *POLD1* provided 85.46% (3063 out of 3584 base pairs) target coverage which included previously published hotspots [12]. Of the 26 coding exons (exons 2–27) in the *POLD1* gene, thirteen of the exons contained missed target coverage (see Supplementary Table 1). PCR enrichment of the 7035 *POLE* and