

BRIEF COMMUNICATION

Massively Parallel Sequencing-Based Clonality Analysis of Synchronous Endometrioid Endometrial and Ovarian Carcinomas

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Abstract

Synchronous early-stage endometrioid endometrial carcinomas (EECs) and endometrioid ovarian carcinomas (EOCs) are associated with a favorable prognosis and have been suggested to represent independent primary tumors rather than metastatic disease. We subjected sporadic synchronous EECs/EOCs from five patients to whole-exome massively parallel sequencing, which revealed that the EEC and EOC of each case displayed strikingly similar repertoires of somatic mutations and gene copy number alterations. Despite the presence of mutations restricted to the EEC or EOC in each case, we observed that the mutational processes that shaped their respective genomes were consistent. High-depth targeted massively parallel sequencing of sporadic synchronous EECs/EOCs from 17 additional patients confirmed that these lesions are clonally related. In an additional Lynch Syndrome case, however, the EEC and EOC were found to constitute independent cancers lacking somatic mutations in common. Taken together, sporadic synchronous EECs/EOCs are clonally related and likely constitute dissemination from one site to the other.

The co-occurrence of adenocarcinoma in the uterus and ovary is found in 5% of endometrial cancer patients and 10% of ovarian cancer patients, and these lesions are largely of endometrioid histology (1,2). Whether these synchronous endometrial and ovarian cancers are two independent primary tumors or metastatic disease has important implications for prognostication and patient management (1,3,4). Despite the adoption of clinical criteria (2,5,6) meant to identify clinically low-risk patients, the distinction between metastatic and independent

primary tumors remains diagnostically challenging. Given that synchronous endometrial and ovarian carcinomas generally present at younger age, earlier stage, and lower grade and have a more favorable prognosis than endometrial or ovarian cancers alone (1–3,7–9), these lesions are often regarded as independent primary tumors rather than advanced-stage metastatic disease (10–12).

Microsatellite instability, immunohistochemistry, loss of heterozygosity, and mutational analyses of single or small sets of

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genes have been used as ancillary markers to help discriminate synchronous primary tumors from metastatic disease (13–17). It should be noted, however, that endometrioid endometrial carcinomas (EECs) and endometrioid ovarian carcinomas (EOCs) harbor similar molecular alterations (18–20), that intratumor genetic heterogeneity has been documented in cancers (21–24), and that the repertoire of genetic alterations in primary tumors and metastases may differ (25,26), which might affect the interpretation of studies based on the analyses of limited numbers of markers/genes. Here, we employed whole-exome and high-depth targeted capture massively parallel sequencing (MPS) to define whether synchronously diagnosed EECs and EOCs, which were clinically defined as either independent primary tumors or metastases, are clonally related.

We collected a series of 23 synchronous EECs and EOCs, which were histologically reviewed by two pathologists (XMG, JCP), subtyped according to the World Health Organization (WHO) criteria (2), and staged and graded according to the International Federation of Gynecology and Obstetrics (FIGO) guidelines (27–30). All samples were anonymized prior to analysis, and approval by the local ethics committees of the respective contributing authors' institutions was obtained. Signed, written informed consent was obtained when appropriate. We extracted DNA from the 23 synchronous EECs and EOCs, eight of which were clinically diagnosed as metastatic disease and 15 as independent primary tumors (Supplementary Table 1, available online), and matched normal DNA from non-neoplastic myometrium or peripheral blood. DNA samples from the first five cases (SYN1–SYN5) were subjected to whole-exome sequencing (WES) (31) to a median depth of 105x (range = 84x–132x) and orthogonal validation using high-depth targeted amplicon resequencing (32). DNA samples from the remaining 18 cases were subjected to MPS targeting all exons and selected introns of 341 ($n = 4$) or 410 ($n = 14$) key cancer genes (MSK-IMPACT [33]) to a median depth of 453x (range = 130x–1484x) (Supplementary Methods and Supplementary Tables 1 and 2, available online).

WES analysis identified a median of 78 nonsynonymous somatic mutations (range = 56–434) in the synchronous EECs (Supplementary Table 3, available online), similar to the number of mutations found in common forms of EECs by The Cancer Genome Atlas (TCGA; median = 71, range = 4–10 860, Mann-Whitney U test $P = .2599$) (34). All synchronous EECs harbored at least one mutation in genes reported to be statistically significantly mutated in common forms of EECs (34) (Supplementary Figure 1 and Supplementary Tables 3 and 4, available online). WES further revealed that the synchronous EECs and EOCs of a given case displayed strikingly similar repertoires of somatic mutations and gene copy number alterations (Figure 1; Supplementary Tables 3 and 5, available online), irrespective of the clinical classification as independent primary or metastatic tumors. Furthermore, synchronous EECs and EOCs shared from 12% to 46% of the somatic mutations identified; however, additional somatic mutations restricted to the EECs or EOCs were identified in each case (Figure 1; Supplementary Tables 1 and 3, available online). We next investigated if the mutational processes that shape the genomes of synchronous EECs and EOCs would differ. Using a previously published approach (Supplementary Methods, available online) (35), we compared the mutational spectra and context of the mutations present in the EECs and EOCs and observed that the mutational processes that have been operative in these lesions did not vary between the tumors from each of the patients analyzed (Figure 1; Supplementary Table 1, available online). We next employed two conservative approaches for clonality analysis, assessing the

likelihood of two samples sharing mutations not expected to have co-occurred by chance, based on all somatic synonymous and nonsynonymous mutations (Supplementary Methods, available online). Both clonality analyses revealed that the EECs and EOCs from each patient were clonally related (Figure 2; Supplementary Figures 2 and 3, available online). These observations suggest that sporadic synchronous EECs/EOCs are clonally related and likely constitute dissemination from one site to the other.

To define whether the differences in the mutational repertoires found in the EEC and EOC from each patient could stem from spatial heterogeneity within these lesions, we obtained three anatomically distinct regions from one EEC analyzed (case SYN4). Truncal mutations (ie, present at high clonal frequencies in all three EEC regions analyzed), including pathogenic mutations affecting *PTEN* and *KRAS*, accounted for 9% of all nonsynonymous somatic mutations; despite the large proportion of branch mutations, the mutational processes did not differ amongst the anatomically distinct areas (Figure 1; Supplementary Table 1, available online), consistent with the notion that EECs may display intratumor spatial heterogeneity, akin to kidney, ovarian, lung, and breast cancers (21–24).

To define the generalizability of our findings, we subjected a series of 17 sporadic synchronous EECs/EOCs and one Lynch Syndrome case to targeted capture MPS (Figure 2A; Supplementary Table 6, available online). Hierarchical clustering of the somatic mutations present in these lesions revealed striking similarities between the EEC and EOC from each patient in all sporadic cases (Supplementary Figure 4, available online). Furthermore, formal analyses of clonal relatedness, based on two statistical approaches, provided evidence to demonstrate that all sporadic EECs and EOCs of a given patient were clonally related (Figure 2B; Supplementary Figures 2 and 3, available online), irrespective of the clinical classification as independent primary or metastatic tumors. In four cases of bilateral EOCs, samples from both EOCs were available and found to be clonally related to each other and their respective EECs (Figure 2B; Supplementary Figures 2 and 3, available online). Importantly, we found that all sporadic synchronous EECs and EOCs from a given patient shared nonsynonymous somatic mutations in at least one cancer driver gene of EEC and/or EOCs, including *PTEN*, *PIK3CA*, *KRAS*, *ARID1A*, or *CTNNB1* (18–20,34) (Supplementary Figure 1 and Supplementary Table 7, available online), in agreement with the findings by Anglesio et al. (36).

In the Lynch Syndrome case (SV2), the EEC and EOC displayed distinct somatic mutations; this case had a disproportionately high number of somatic mutations, and the patient was found to harbor a germline *MSH6* mutation (p.R1076C) (Figure 2C). The EEC and EOC samples from this patient harbored distinct somatic *MSH6* loss-of-function mutations in each site and lacked *MSH6* expression (Figure 2C; Supplementary Table 6, available online); furthermore, the EEC but not the EOC harbored a somatic *POLE* p.S459F hotspot mutation and displayed a mutational signature consistent with that of a hereditary ultra-hypermutated EEC (ie, an EEC with a germline mismatch repair gene mutation and a somatic *POLE* mutation) (35,37,38).

This study has important limitations. Our data provide strong evidence to suggest that in patients with sporadic synchronous EECs/EOCs these lesions are clonally related and likely constitute dissemination from one site to the other. Based on the limited sample size and approach employed, however, we can neither provide direct evidence to infer the chronology of the development of the endometrial and ovarian tumors in patients with synchronous EECs/EOCs nor define the biological basis of the metastatic



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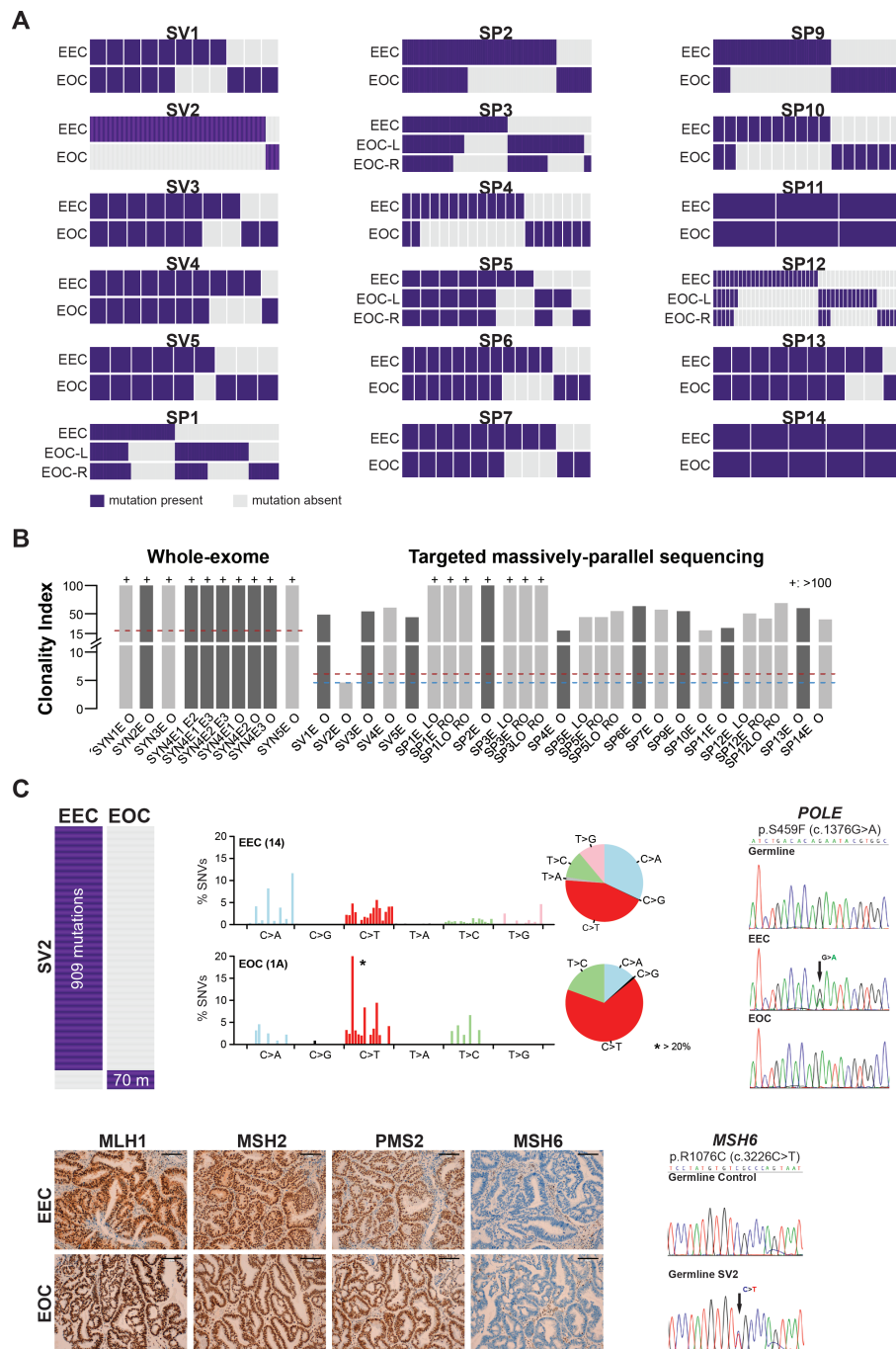


Figure 2. Clonal relatedness analysis of synchronous endometrioid endometrial carcinomas (EECs) and endometrioid ovarian carcinomas (EOCs) occurring in a sporadic or Lynch Syndrome context. **A)** Nonsynonymous and synonymous somatic single-nucleotide variants and small insertions/deletions identified by targeted MPS analysis in 18 cases of synchronous EECs/EOCs. Note that in all cases but SV2 the EEC and the EOC shared somatic mutations. **B)** Clonality Index (CI2) for the 23 cases of synchronous EECs/EOCs analyzed in this study, defined as the likelihood of two lesions sharing mutations not expected to have co-occurred by chance (Supplementary Methods, available online). Red dotted lines indicate the threshold to define clonal relatedness for the respective sequencing platform (whole-exome sequencing left, targeted capture massively parallel sequencing right). Blue dotted line indicates the CI2 at which two samples from a given patient did not share any mutation on the respective sequencing platform. With the exception of case SV2, a Lynch Syndrome case, all tumors from a given patient were found to be clonally related. **C)** In SV2, MPS analysis demonstrated that none of the somatic mutations were shared between the synchronous EEC and EOC. The mutational signatures of the nonsynonymous and synonymous somatic single-nucleotide variants in the EEC and EOC of SV2 are displayed according to the 96 substitution classification defined by the substitution classes (C>A, C>G, C>T, T>A, T>C, and T>G bins) and the 5' and 3' sequence context. All mutational signatures are normalized to the trinucleotide frequency of the human genome. The EEC displayed a mutational signature consistent with that of a hereditary ultra-hypermutated carcinoma (ie, a tumor with a germline mismatch repair gene mutation and a somatic POLE hotspot mutation), whereas the EOC displayed a mutational signature related to aging (35,37,38). A POLE S459F hotspot mutation was identified in the EEC but not in the synchronous EOC as shown in the sequence electropherograms. Both the EEC and the synchronous EOC displayed loss of MSH6 expression as assessed by immunohistochemistry (scale bar = 100 μ m). The patient SV2 harbored a germline MSH6 (R1076C) mutation as shown in the sequence electropherograms. EEC = endometrioid endometrial carcinoma; EOC = endometrioid ovarian carcinoma; m = mutations; SNV = single-nucleotide variant.

route in these patients. It is plausible that the favorable prognosis of most of these patients might be explained by the fact that the EOCs represent ovarian implants of likely indolent EECs (eg, small tumor size, low/intermediate grade, and/or tumors predominantly composed of complex atypical endometrial hyperplasia) and that these implants might occur as a result of retrograde flux from the uterine corpus through the fallopian tubes rather than hematogenous/lymphatic metastatic spread. Further studies to define the chronology of the development of these synchronously diagnosed, clonally related cancers and the biological basis for the presence of uterine and ovarian disease, but no peritoneal spread, are warranted. Given these uncertainties, one could contend that despite their clonal relatedness at present patients with synchronous EEC/EOC should be managed following current guidelines based on clinico-pathologic criteria (2,3,5,6). Our results, however, support the development of prospective clinical trials to define the optimal treatment for patients with synchronously diagnosed EECs/EOCs, which cannot be definitely classified into low-/high-risk groups based on current criteria.

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Notes

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Accession code: The whole-exome and targeted massively parallel sequencing data have been deposited into the National Center for Biotechnology Information Sequence Read Archive under accession codes SRP059545 and SRP059544, respectively.

The authors have no conflicts of interest to declare.

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