

## BRIEF COMMUNICATION

# Synchronous Endometrial and Ovarian Carcinomas: Evidence of Clonality

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## Abstract

Many women with ovarian endometrioid carcinoma present with concurrent endometrial carcinoma. Organ-confined and low-grade synchronous endometrial and ovarian tumors (SEOs) clinically behave as independent primary tumors rather than a single advanced-stage carcinoma. We used 18 SEOs to investigate the ancestral relationship between the endometrial and ovarian components. Based on both targeted and exome sequencing, 17 of 18 patient cases of simultaneous cancer of the endometrium and ovary from our series showed evidence of a clonal relationship, ie, primary tumor and metastasis. Eleven patient cases fulfilled clinicopathological criteria that would lead to classification as independent endometrial and ovarian primary carcinomas, including being of FIGO stage T1a/1A, with organ-restricted growth and without surface involvement; 10 of 11 of these cases showed evidence of clonality. Our observations suggest that the disseminating cells amongst SEOs are restricted to physically accessible and microenvironment-compatible sites yet remain indolent, without the capacity for further dissemination.

Synchronous endometrial and ovarian cancers (SEOs) have been reported in 5% to 10% of endometrial or ovarian cancers (1,2). When organ confined and low grade, SEOs behave as if they were two independent primary tumors rather than an advanced-stage carcinoma of either ovary or endometrium. Methods of defining metastatic vs independent primary tumors have led to controversy regarding the relatedness of SEOs. Clinical features, in particular a very favorable prognosis, suggest that the majority of low-stage SEOs are independent primary tumors (2–6). If most low-stage SEOs were shown to be

clonally related, this would be evidence of a metastatic event that, unlike typical metastasis, has little impact on prognosis.

We sought to examine SEOs through targeted sequencing of 35 genes commonly altered in endometrial (7–11) and ovarian (8–10,12–14) cancers to establish whether there is a clonal lineage between SEOs that, based on clinical features, are predicted to be independent tumors (*Supplementary Table 1*, available online). Eighteen SEOs from the Vancouver General Hospital (VGH; n = 15) and the University Hospital Tuebingen (TBG; n = 3) were reviewed, confirming histopathological diagnosis, grade, and stage (*Table 1*;

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**Table 1.** Cohort details\*

Case ID	Source	Age, y	Ovarian STAGE	Ovarian histotype	Uterine STAGE	Uterine histotype	Exome	Class
SEO_TBG_15	TBG	45	1A	ENOC	T1a	ENEC		Synchronous
SEO_TBG_22	TBG	46	3B	CCOC	T1b	ENEC		independent
SEO_TBG_31	TBG	66	1A	ENOC	T1a	ENEC		(SI)
SEO_VAN_08	VGH	37	1A	ENOC	T1a	ENEC		
SEO_VAN_14	VGH	76	1A	ENOC	T1a	ENEC		
SEO_VAN_22	VGH	61	1A	ENOC	T1a	ENEC		
SEO_VAN_27	VGH	42	1A	ENOC	T1a	ENEC	YES	
SEO_VAN_29	VGH	49	1A	ENOC	T1a	ENEC		
SEO_VAN_43	VGH	50	1A	ENOC	T1a	ENEC	YES	
SEO_VAN_54	VGH	49	1C	CCOC	T1a	ENEC	YES	
SEO_VAN_60	VGH	39	1A	ENOC	T1a	ENEC	YES	
SEO_VAN_04	VGH	66	1C	ENOC	T2	ENEC		Metastatic (M)
SEO_VAN_07	VGH	61	1C	ENOC	T1a	ENEC		
SEO_VAN_33	VGH	46	1C	ENOC	T1a	ENEC		
SEO_VAN_40	VGH	50	1C	ENOC	T1a	ENEC		
SEO_VAN_56	VGH	47	2C	ENOC	T1a	ENEC		
SEO_VAN_58	VGH	54	1C	ENOC	T1a	ENEC		
SEO_VAN_65	VGH	54	1C	ENOC	T1a	ENEC	YES	

\* FIGO staging criteria and nomenclature is used for ovarian and/or endometrial carcinomas. CCOC = Clear Cell Ovarian Carcinoma; ENEC = Endometrioid Endometrial Carcinoma; ENOC = Endometrioid Ovarian Carcinoma; TBG = University Hospital Tuebingen; VGH = Vancouver General Hospital.

FIGO Grade and ovarian stage; TNM for endometrial cancer stage [15]). Consent, or waiver of consent, was obtained for all specimens at contributing hospitals while the study was approved under the University of British Columbia and BC Cancer Agency research ethics board. See the [Supplementary Methods](#) (available online) for detailed sample information. Representative hematoxylin and eosin (H&E) sections from each SEO pair are presented in [Supplementary Figure 1](#) (available online). Nine patient cases of endometrioid histotype, at both ovarian (endometrioid ovarian carcinoma [ENOC]) and endometrial (endometrioid endometrial carcinoma [ENEC]) sites, were considered independent primaries based on published criteria (3,15,16). Two patient cases had a different histotype diagnosis for the ovarian (clear cell ovarian carcinoma [CCOC]) and endometrial (ENEC) tumors and were also considered independent primaries. The remaining seven patient cases, were indeterminate or suspected to be metastatic based on clinical features. For simplicity, we grouped the prior 11 cases as suspected synchronous independent (SI) and the latter seven as suspected metastasis (M).

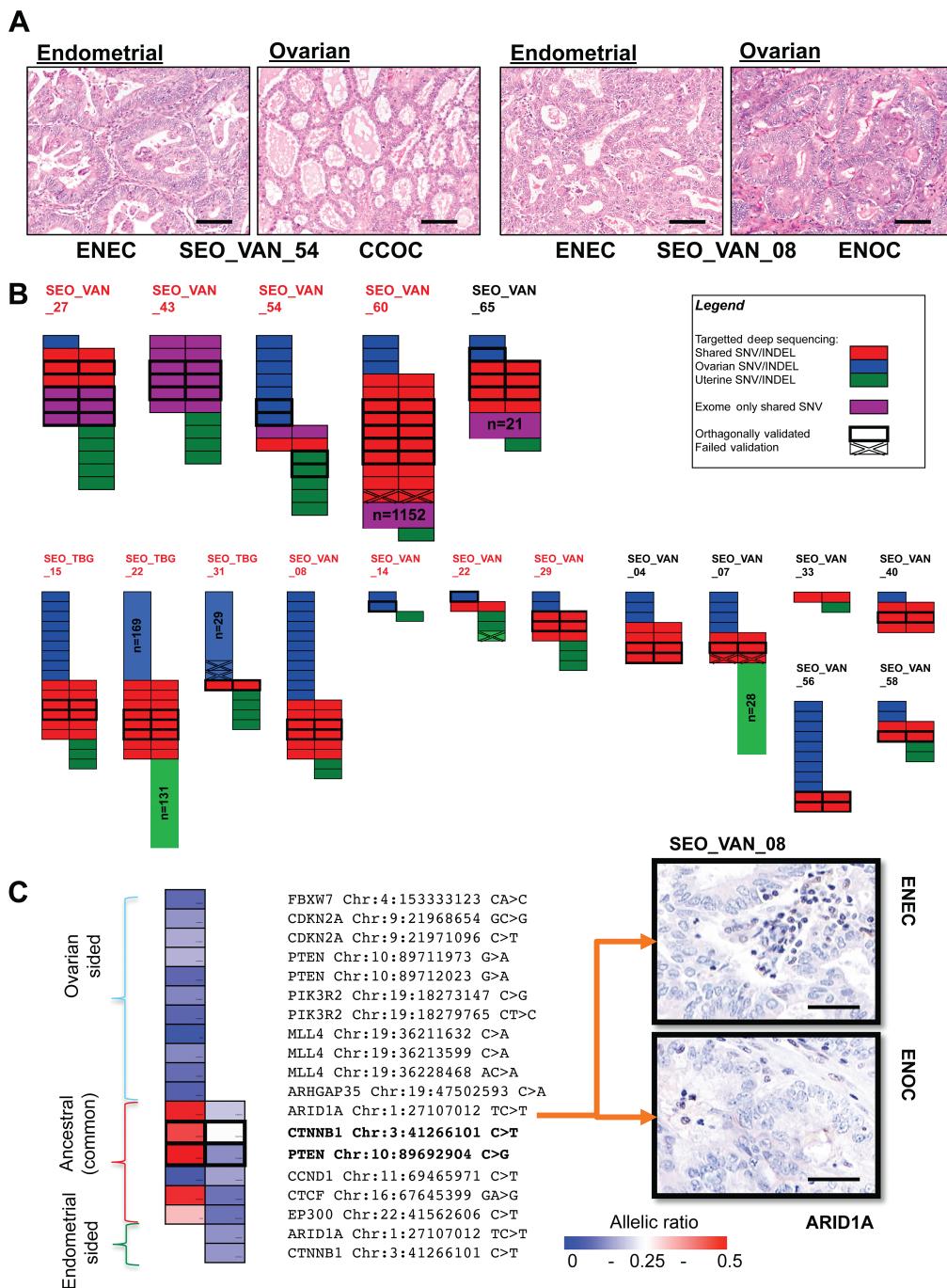
We report somatic single-nucleotide variants (SNVs) and insertion/deletions (INDELs) discovered in targeted deep sequencing; refer to the [Supplementary Methods](#) (available online) for details on all methods used in the analysis. SNVs ([Supplementary Table 2](#), available online) are restricted to COSMIC (17) and non-COSMIC variants, where the latter were not formalin-fixed, paraffin-embedded (FFPE) artefact-associated transitions (C>T [18]). Likewise, INDELs were filtered to include only those with a higher than 10% allelic ratio ([Figure 1; Supplementary Table 2](#), available online). All but two patient cases showed evidence for at least one shared (identical position and base change) variant between the ovarian and endometrial pair. Exome data was available for five patient cases; four of the five showed multiple concordant shared mutations between exome and deep sequencing ([Supplementary Table 3](#), available online) after filtering as noted above. DNA copy number and allelic frequency plots generated from exome data were consistent with clonal copy number and LOH changes in three cases ([Supplementary Figure 2](#), available online); two cases were uninformative. Finally, a subset of positions was manually selected for orthogonal Sanger sequencing validation from both pools of targeted and exome-derived variants ([Figure 1](#);

[Supplementary Table 4](#), available online). After incorporation of targeted and exome-derived data, we were left with a single SI case without evidence of clonality (SEO\_VAN\_14). Within the targeted data (postfilter), the number of identical shared mutations in SI and M varied (range = 0–9 and 1–4, respectively) ([Figure 1](#)), with no statistical difference detectable between groups ( $P = .39$ , two-tailed Student's t test).

None of the genes we examined displayed shared variants corresponding to prototypical single-hotspot change cancer genes (such as those from BRAF/KRAS), arguing against a convergent evolution process. Two SI patient cases, SEO\_VAN\_60 and SEO\_TBG\_22, had a very large number of variants even after conservative filtering. SEO\_VAN\_60 carried two POLE mutations in the ancestral pool, while both endometrial and ovarian tumors of SEO\_VAN\_22 each carried (nonidentical) POLE mutations. It is plausible that this may represent a hypermutator phenotype, as has been described for endometrial cancers harboring POLE mutations (11,19,20). Our mutation data strongly support a clonal lineage, and therefore a common ancestral clone, in all but one SI-SEO. This is highly concordant to a parallel, independent study from Schultheis et al. (21), wherein a clonal relationship between SEOs was also observed in 22 of 23 patient cases.

Previous studies have attempted to use mutational analysis to examine the relatedness of synchronous ovarian and endometrial tumors and have suggested that only a fraction of so-called synchronous independent primary tumors have an ancestral relationship (2–6). Such studies have been hampered by the examination of single genes or low-resolution cytogenetics, leaving most cases uninformative. Our gene panel, although still restricted, was sufficiently informative to show the majority of patient cases in our series are clonally related and thus not independent primary tumors. It should be noted that we also observed a number of mutations unique to either the ovarian or endometrial carcinomas (targeted sequencing, postfilter mean = 12, median = 2). This may suggest divergence at each site with resulting intratumoral heterogeneity (22,23). With regards to the true primary anatomic site of these malignancies, it was not possible, based on our data or that of Schultheis et al. (21), to conclusively ascertain the directionality.

Gynecological cancers show a predilection for locoregional metastasis; this is especially true for ovarian cancer



**Figure 1.** Sequencing-based profiles of synchronous ovarian and endometrial carcinomas. **A)** Hematoxylin and eosin (H&E) images from two prototypical patient cases of synchronous-independent (SI) primary carcinomas of ovary and endometrium, based on clinicopathological criteria. SEO\_VAN\_54 is composed of a clear cell ovarian carcinoma (CCOC) while the corresponding endometrial tumor is of endometrioid histotype (ENEC). SEO\_VAN\_08 is more typical, showing endometrioid histology at both sites (ENEC and ENOC, respectively). Scale bar = 100 µm. **B)** Display of targeted deep sequencing-derived somatic mutations detected in synchronous ovarian (left column) and endometrial (right column) carcinomas are represented by colored bars (see legend in graphic). Ancestral (shared) variants from exome data after filtering and in the first five patient cases only are also shown. Those defined clinically as synchronous-independent primary carcinomas (case numbers in red, n = 7) are indistinguishable from those that were classified as possibly metastatic (case numbers in black, n = 7). For the purpose of display, cases with more than 15 somatic variants in any one category are presented with an out-of-scale block showing the total number of somatic variants. Variants were considered orthogonally validated if present in at least two platforms (denoted by bold outlines) (Supplementary Table 4, available online). Variants where an attempt to validate by Sanger sequencing failed to show a (somatic) nucleotide change are marked with a double X in corresponding cells. The most frequently altered genes among the identical shared mutations based on targeted sequencing was PTEN (14 alterations in 9 cases), followed by CTNNB1 (9 alterations in 9 cases). Identical mutations in samples from different patients were seen only twice: SEO\_VAN\_04 and SEO\_VAN\_08 shared identical PTEN R130G mutations while SEO\_VAN\_29 and SEO\_VAN\_60 shared PTEN R130Q mutations. In these instances, additional (case-specific) shared variants were detectable in all except SEO\_VAN\_04 (Supplementary Tables 2–4, available online). **C)** Detailed information on SEO\_VAN\_08 showing somatic variants detectable in our targeted deep sequencing panel including ancestral mutations in CTNNB1, PTEN, and ARID1A. The ARID1A frame-shift mutation, observed at an allelic ratio of 0.42 and 0.25 in ovarian and endometrial tumors respectively, can be seen to result in loss of nuclear ARID1A protein in carcinoma cells from both sites (but not stromal cells, which serve as an internal positive control) by immunohistochemistry. Scale bar = 30 µm. Sanger sequencing was used for orthogonal validation of CTNNB1 and PTEN variants (bold).

(24–26), where transcoelomic spread with extensive peritoneal involvement and no evidence of distant metastasis is common at the time of presentation. The metastatic behavior of SEOs is much more restricted, with exclusive involvement of endometrial and ovarian sites. The ability to cure extra-organ metastasis routinely through surgery alone, without adjuvant chemotherapy or radiation therapy, sets SEOs clinically apart from other examples of loco-regional metastasis. Dissemination may not be dependent on full transformation, and this incomplete metastatic phenotype underlies the favorable prognosis of SEOs.

This phenomenon of restricted dissemination may be applicable to a broader spectrum of tumors where cells have the ability to detach from a primary lesion without undergoing apoptosis (anoikis), spreading through (open) spaces, and recolonizing only exclusive microenvironments without widespread metastasis. We propose this microenvironment restriction is the dominant trait that sets SEOs apart from ovarian carcinomas with transcoelomic spread to multiple sites, associated with a poor prognosis. Low-grade carcinomas, including HPV-associated cervical/vulvar and cervical/ovarian carcinoma as well as low-grade urothelial carcinomas (27–29), may exhibit similar restricted spread. In such examples, spread beyond the primary site is not associated with distant metastasis in most patients and, most importantly, continues to be associated with surgical resectability and favorable outcome. This scenario of clinically indolent spread is sufficiently common to warrant recognition, as it is important not to overtreat patients when surgical removal of specific metastatic or “pseudo-metastatic” foci can lead to cure. A similar process may also be relevant to benign precursors and borderline tumors; of potential biological relevance to SEOs, recent data on ovarian cancer-associated endometriosis have shown a clonal relationship between the ovarian carcinoma and foci of benign endometriosis distant from the carcinoma (30).

Even in consideration of the limited cohort size and restricted sequencing panel, taking account the identical results of Schultheis et al. using independent series and analysis strategy (21), our findings conclusively demonstrate that the majority of SEOs are clonally derived. When considering individual mutations from deep sequencing and exome data, caution should be exercised, given a source material (FFPE) prone to sequencing artifacts. As such, we do not believe this dataset is well suited to discovery of somatic mutation critical to driving divergence between ovarian and endometrial sites. Clearly there remain many unanswered questions, such as site of origin and directionality of metastasis, as well as the specific ovarian and endometrial microenvironment features that may influence progression. Further study of SEOs is needed to address these issues and provide insight into the minimal complement of genetic/epigenetic events required to initiate extra-organ metastasis.

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MSA and SK conceived the project with oversight from DGH and CBG. MM, JS, RM, and MSA carried out all wet-bench experiments. MM, SK, and MSA selected samples with pathology review by HMH, HLC, ANK, BSS, FK, AS, and CBG. FAT, AS, SB, FK, DW, and SK provided oversight for sample acquisition from University Hospital Tuebingen. MM, MKM, and MSA designed the deep-sequencing target panel. YKW, AB, DSG, and SPS provided bioinformatics support. Interpretation of sequencing, copy number, and LOH data were done by MSA, YKW, and AB. MSA, CBG, and HMH wrote the initial draft of the manuscript; all authors approved the final version.

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