

This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.

Author(s): Niskakoski, Anni; Pasanen, Annukka; Porkka, Noora; Eldfors, Samuli; Lassus, Heini; Renkonen-Sinisalo, Laura; Kaur, Sippy; Mecklin, Jukka-Pekka; Bützowb, Ralf; Peltomäki, Päivi

Title: Converging endometrial and ovarian tumorigenesis in Lynch syndrome : shared origin of synchronous carcinomas

Year: 2018

Version: Published version

Copyright: © 2018 The Authors. Published by Elsevier Inc.

Rights: _{CC BY 4.0}

Rights url: https://creativecommons.org/licenses/by/4.0/

Please cite the original version:

Niskakoski, A., Pasanen, A., Porkka, N., Eldfors, S., Lassus, H., Renkonen-Sinisalo, L., Kaur, S., Mecklin, J.-P., Bützowb, R., & Peltomäki, P. (2018). Converging endometrial and ovarian tumorigenesis in Lynch syndrome : shared origin of synchronous carcinomas. Gynecologic Oncology, 150(1), 92-98. https://doi.org/10.1016/j.ygyno.2018.04.566



Contents lists available at ScienceDirect

Gynecologic Oncology



journal homepage: www.elsevier.com/locate/ygyno

Converging endometrial and ovarian tumorigenesis in Lynch syndrome: Shared origin of synchronous carcinomas



Anni Niskakoski ^{a,*}, Annukka Pasanen ^b, Noora Porkka ^a, Samuli Eldfors ^c, Heini Lassus ^d, Laura Renkonen-Sinisalo ^e, Sippy Kaur ^{a,f}, Jukka-Pekka Mecklin ^{g,h}, Ralf Bützow ^{b,d}, Päivi Peltomäki ^a

^a Department of Medical and Clinical Genetics, University of Helsinki, Helsinki, Finland

^b Department of Pathology, University of Helsinki and HUSLAB, Helsinki University Hospital, Finland

^c Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland

^d Department of Obstetrics and Gynecology, University of Helsinki, Helsinki University Hospital, Finland

^e Second Department of Surgery, Helsinki University Central Hospital, Helsinki, Finland

^f Department of Oral and Maxillofacial diseases, University of Helsinki and Helsinki University Hospital, Finland

^g Department of Surgery and Education & Science, Central Finland Health Care District, Finland

^h Department of Sport and Health Sciences, Jyväskylä University, Finland

HIGHLIGHTS

· Synchronous gynecological carcinomas from Lynch syndrome are molecularly concordant, suggesting shared origins.

· Complex hyperplasias without or with atypia molecularly resemble endometrial and ovarian carcinomas from the same patients.

· Joint involvement of endometrium and ovaries needs to be taken into account in clinical management of Lynch syndrome.

ARTICLE INFO

Article history: Received 13 March 2018 Received in revised form 19 April 2018 Accepted 20 April 2018 Available online 30 April 2018

Keywords: Lynch syndrome Endometrial cancer Ovarian cancer Endometrial hyperplasia Hypermethylation Mismatch repair

ABSTRACT

Objective. The diagnosis of carcinoma in both the uterus and the ovary simultaneously is not uncommon and raises the question of synchronous primaries vs. metastatic disease. Targeted sequencing of sporadic synchronous endometrial and ovarian carcinomas has shown that such tumors are clonally related and thus represent metastatic disease from one site to the other. Our purpose was to investigate whether or not the same applies to Lynch syndrome (LS), in which synchronous cancers of the gynecological tract are twice as frequent as in sporadic cases, reflecting inherited defects in DNA mismatch repair (MMR).

Methods. MMR gene mutation carriers with endometrial or ovarian carcinoma or endometrial hyperplasia were identified from a nationwide registry. Endometrial (n = 35) and ovarian carcinomas (n = 23), including 13 synchronous carcinoma pairs, were collected as well as endometrial hyperplasias (n = 56) and normal endometria (n = 99) from a surveillance program over two decades. All samples were studied for MMR status, ARID1A and L1CAM protein expression and tumor suppressor gene promoter methylation, and synchronous carcinomas additionally for somatic mutation profiles of 578 cancer-relevant genes.

Results. Synchronous carcinomas were molecularly concordant in all cases. Prior or concurrent complex (but not simple) endometrial hyperplasias showed a high degree of concordance with endometrial or ovarian carcinoma as the endpoint lesion.

Conclusions. Our investigation suggests shared origins for synchronous endometrial and ovarian carcinomas in LS, in analogy to sporadic cases. The similar degrees of concordance between complex hyperplasias and endometrial vs. ovarian carcinoma highlight converging pathways for endometrial and ovarian tumorigenesis overall. © 2018 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

* Corresponding author at: Biomedicum Helsinki, Department of Medical and Clinical Genetics, 00014 Helsinki, Finland.

E-mail address: anni.niskakoski@helsinki.fi (A. Niskakoski).

Endometrial and ovarian carcinomas are among the most common female cancers in the Western world. In the United States, >60,000 and 20,000 new cases, respectively, are expected to be diagnosed in 2018 [1]. Among gynecologic cancers, endometrial cancer is the most

https://doi.org/10.1016/j.ygyno.2018.04.566

0090-8258/© 2018 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

prevalent, whereas ovarian cancer is the leading cause of death. Endometrial and ovarian cancer may occur as part of Lynch syndrome (LS), in which inherited defects in DNA mismatch repair (MMR) underlie autosomal dominantly inherited predisposition to cancers of multiple organs [2]. While colorectal cancer is the most common cancer in LS overall, the incidence of endometrial cancer equals to or even exceeds that of colorectal cancer in female carriers of MMR gene mutations [3,4]. Up to 54% and 24% of female mutation carriers develop endometrial and ovarian cancer, respectively, at some point of their lives [3,4]. On the population level, 9% of endometrial cancer cases under 50 years of age [5] and 2% of ovarian cancer cases unselected for age [6] have been estimated to be due to germline mutations in MMR genes. Endometrial cancer in LS is of endometrioid histology in ~90% of cases and associated with earlier age at diagnosis (mean 50 vs. 68 years) and a higher prevalence of lower uterine segment involvement compared to sporadic cases [7,8]. Ovarian cancer in LS is likewise diagnosed at a younger age (mean 45 years, which is 15-20 years earlier than in sporadic cases), and 77% of epithelial ovarian carcinomas in LS are non-serous [9] in a marked contrast with the average population where the high-grade serous type predominates [10].

In 10% of sporadic cases [11] and 20% of LS cases [7,12], carcinomas are diagnosed in both the uterus and the ovary simultaneously, raising the question of tumor origins: do the two cancers arise independently or one as a metastasis of the other? In the sporadic setting, two recent studies addressed this question by targeted sequencing, and shared profiles of somatic mutations suggested that synchronous tumors represented metastatic disease from one site to the other [13,14]. However, synchronous endometrial and ovarian carcinomas from an additional LS case lacked somatic mutations in common, implying that LS might constitute an exception to the general rule [14]. Epidemiological observations suggest that the developmental pathways to endometrial and ovarian carcinoma may cross far prior to malignant transformation. Up to 42% of women in whom endometrial sampling reveals atypical endometrial hyperplasia are found to have simultaneous endometrial cancer in hysterectomy specimens [15] consistent with the idea that endometrioid endometrial carcinoma evolves via endometrial hyperplasia [16]. Interestingly, some 50% of patients with endometrioid ovarian carcinoma, too, display concurrent atypical endometrial hyperplasia [17], the significance of which remains to be clarified: does endometrial hyperplasia represent an early step of synchronous endometrial tumorigenesis or have relevance for ovarian cancer development as well, given that endometrial epithelial cells are considered to be the origins of endometrioid and clear cell carcinomas of the ovary [18]?

We took advantage of synchronous cancers arising in LS individuals and consecutive endometrial biopsy specimens from lifelong surveillance of MMR gene mutation carriers to examine the relationship between endometrial and ovarian tumorigenesis. Our results define the developmental routes of endometrial and ovarian cancer and are clinically relevant.

2. Materials and methods

2.1. Patients and samples

The nation-wide Hereditary Colorectal Cancer Registry of Finland was used as a source to identify LS individuals with endometrial or ovarian carcinoma or endometrial hyperplasia. Tumor and preceding surveillance specimens were available from 66 mutation carriers (*MLH1* 52, *MSH2* 10, and *MSH6* 4), including a total number of 213 samples (Supplementary Table S1). Endometrial hyperplasia specimens were classified into four categories (simple hyperplasia, SH; simple atypical hyperplasia, SAH; complex hyperplasia without atypia, CH; and complex hyperplasia with atypia, CAH) in accordance with the WHO1994/ 2003 classification, since it was the original schema used in sample diagnostics [19,20]. A category including SAH was omitted because only one SAH sample was identified. A gynecological pathologist had originally determined the histology of specimens and the diagnosis was verified after sample collection by a gynecological pathologist (R.B.). Hematoxylin and eosin was used to stain formalin-fixed paraffin-embedded (FFPE) tissue sections for visual inspection and tumor sections containing >60% of tumor cells were chosen for DNA extraction performed by a customized protocol [21]. Manual microdissection was used to carefully separate normal, hyperplasia and tumor samples. The study was approved by the Institutional Review Boards of the Departments of Surgery (466/E6/01) and the Obstetrics and Gynecology (040/95) of the Helsinki University Central Hospital (Helsinki, Finland) and Jyväskylä Central Hospital (Jyväskylä, Finland) (Dnro 5/2007). The National Supervisory Authority for Welfare and Health (Valvira/Dnro 10741/06.01.03.01/2015) approved the collection of archival samples.

2.2. Immunohistochemistry (IHC) for L1CAM and ARID1A

PT-Module (Lab Vision, CA, USA) was obtained to perform antigen retrieval on 4 µm deparaffinized tissue slides at 98C°/20 min in Envision TM Flex Target Retrieval solution pH 9 for L1CAM and pH 6.1 for ARID1A (Agilent Technologies, USA). The antibodies used were Covance SIG-39110-200 produced in mouse for L1CAM (1:40/20 min, CD171, clone 1E11, Covance) and anti-ARID1A antibody produced in rabbit (1:200/20 min, HPA005456, polyclonal, Lot D104841, Sigma-Aldrich, USA). Slides were stained with Autostainer 480 automated immunostainer (Lab Vision, CA, USA) and hematoxylin (Mayers HTX, Histolab) was used to counterstain tissue sections. Protein expression was evaluated and scored from stained slides by two pathologists (R.B. and A.P.). Membranous L1CAM staining of cells was scored as positive/abnormal when >10% of tumor cells expressed L1CAM. ARID1A expression was scored as negative/abnormal when all tumor cell nuclei stained negative but positive expression was preserved in stromal cells.

2.3. Mismatch repair (MMR) status

Sample DNA was investigated by polymerase chain reaction (PCR) using fluorescently labeled mononucleotide repeat markers BAT25 and BAT26. If both markers were stable, the interpretation was microsatellite stability (MSS), whereas one or two unstable markers indicated microsatellite-instability (MSI) [22]. Immunohistochemistry (IHC) was performed to investigate MMR protein expression as described [23]. MMR was regarded deficient by the presence of MSI, absence of MMR protein, or both.

2.4. Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA)

Samples were investigated using methylation-specific (MS)-MLPA SALSA MLPA ME001-C2 test (MRC-Holland, Amsterdam, The Netherlands) as described [23] to analyze methylation patterns of 24 general tumor suppressor genes (TSGs) (listed at http://www.mrcholland.com) often methylated in several cancers. In addition, custom designed MS-MLPA probe mix including 7 gene probes supplemented with Salsa MLPA kit P-300-B1 human DNA reference-2 reagents was used to analyze methylation alterations in genes often methylated specifically in endometrial and ovarian cancer as described [24]. The test produces a methylation dosage ratio (Dm), which varies between 0 and 1.0 and reflects the percentage of methylated DNA. The Dm value was calculated individually for each sample as previously described [25]. The Dm value of 0.15 or above was set as the technical threshold for indication of hypermethylation for all genes included in the 24 TSG MS-MLPA test [25], except for CDKN2B. The hypermethylation thresholds for each of the seven endometrial and ovarian carcinoma-related genes included in the custom MS-MLPA test and for CDKN2B included in the commercial test were determined using LS normal endometrial

samples and calculated as the mean Dm in normal endometria plus 1 standard deviation.

2.5. Targeted sequencing for somatic mutations

Tumors and corresponding normal DNA samples were sequenced at the Institute for Molecular Medicine Finland (FIMM; Helsinki, Finland). Sequencing was performed on Illumina HiSeq 2500 platform (San Diego, CA) using the Nimblegen Comprehensive Cancer Panel (Roche Diagnostics), a 4 Mb design with 578 cancer-related genes, as described [26]. In brief, libraries were prepared using ThruPLEX® DNA-seq Kit, and the exons captured according to the manufacturer's protocol (Rubicon Genomics). The mean target coverage for tumors was 106-fold (Supplementary Table S2). The variant calling pipeline is described in Sulonen et al. [27]. VarScan 2 mutation detection algorithm version 2.3.2 was used identification of the non-synonymous somatic mutations from the paired normal and tumor data [28] as described [26]. Variants with VarScan somatic *p*-value below 0.01 were selected for subsequent analyses.

VarSeq (GoldenHelix®) was used to conduct in silico evaluation of somatic single nucleotide variants. The algorithms used to predict the effect of amino acid substitution on protein function were SIFT, PolyPhen-2, MutationTaster, MutationAssessor, FATHMM, and FATHMM MKL Coding. Mutation ID in COSMIC was provided if the mutation was present in the Catalogue of somatic mutations in cancer (COSMIC v71, GRCh 37; http://grch37-cancer.sanger.ac.uk/cosmic).

2.6. Statistical analyses

SPSS software version 22.0 (IBM® SPSS® Statistics, Inc. Chicago, IL, USA) was used for statistical evaluations. Frequency data was analyzed by Fisher's exact test. Shapiro-Wilk test was performed to test normality of data. Comparisons between two groups including numbers of methylated genes or Dm values were evaluated by the Student's *t*-test (for normally distributed samples) or nonparametric Mann-Whitney *U* test (for not normally distributed samples). *p* values < 0.05 (2-tailed) were considered significant.

3. Results

3.1. Study rationale and frequencies of molecular alterations

Prompted by the discordant preliminary observations between sporadic and LS-associated synchronous carcinomas [13,14], we undertook this study to explore the relationship between endometrial and ovarian tumorigenesis in LS. Our investigation is based on 213 specimens from 66 carriers of MMR gene mutations and includes carcinomas of the endometrium (endometrioid) and ovary (endometrioid and clear cell), as well as consecutive specimens of non- and premalignant endometrial tissues from a surveillance program operative since 1996 [29] (Supplementary Table S1). We recently used this series to investigate the frequencies of MMR, ARID1A, and TSG methylation alterations against the progressive histological abnormality of endometrial specimens from LS and sporadic cases [30]. We now focus on synchronous carcinomas (13 pairs from equally many individuals) and endometrial hyperplasia – endometrial/ovarian carcinoma combinations (35 pairs from 22 mutation carriers) to explore their clonal relatedness. LS synchronous carcinomas are additionally compared by targeted sequencing. All LS specimens are investigated for the protein expression of L1CAM, an adhesion molecule connected to invasion and metastatic potential [31–33], to supplement our previous set of markers [30] monitoring early alterations.

Table 1 shows the frequencies of molecular changes detected in the LS sample series (this study and [30]). Compared to the very high frequencies (up to 100%) for MMR defects and ARID1A expression loss, LICAM aberrations were less prominent. The highest frequencies were seen in ovarian clear cell carcinomas (OvCC), of which 43% (3/7) displayed L1CAM overexpression. Representative examples of immunohistochemical staining results of L1CAM are given in Supplementary Fig. S1.

3.2. Pairwise evaluation of synchronous gynecological carcinomas for concordance

Our LS series included 13 pairs of synchronous carcinomas (9 endometrial plus ovarian carcinoma pairs, 3 cases with bilateral ovarian carcinomas, and one pair with endometrial and endocervical carcinoma). Fig. 1 depicts case by case the molecular alterations discovered in the tumors. To systematically compare the paired tumors for concordance, evaluation criteria were developed taking 5 parameters (MMR status, ARID1A protein expression, L1CAM protein expression, hypermethylation status of 7 endometrial and ovarian cancer-related TSGs and hypermethylation status of 24 general TSGs) into account (please see footnote of Fig. 1). A pair was regarded concordant if at least 3 of 5 (or at least 50%) of parameters were concordant. By these criteria, all synchronous cases were deemed concordant. Synchronous tumors invariably shared the same MMR and ARID1A expression status, and TSG hypermethylation patterns also exhibited high intra-pair concordance (Fig. 1). Our findings thus suggested that the synchronous LS cancers had shared origins (i.e., in each pair, one tumor was likely to be a metastasis of the other).

To further explore the relationship between those synchronous carcinomas that affected different organs (the ovary and the endometrium), the 9 synchronous ovarian and endometrial carcinomas were statistically compared as groups relative to each other and to nonsynchronous ovarian and endometrial carcinomas (Table 2). As molecular parameters, the frequencies of MMR, ARID1A, and L1CAM alterations and the numbers of hypermethylated TSGs (representing two gene panels), and the methylation dosage ratios (Dm values) for 6 genes most commonly methylated in our series (*RSK4, SPARC, HOXA9, HOXA10, RASSF1* and *CDH13*) were considered. No significant differences between the synchronous tumors were found (Fisher's exact test was used for frequencies and paired *t*-test for Dm values). Some significant differences were observed in synchronous vs. non-synchronous

Table 1

Molecular alterations in endometrial and ovarian carcinomas and in non- and pre-malignant endometrial specimens.

	Endometrial endometrioid carcinoma (EnCa)	Ovarian clear cell carcinoma (OvCC)	Ovarian endometrioid carcinoma (OvE)	Normal endometrium	Simple hyperplasia (SH)	Complex hyperplasia without atypia (CH)	Complex atypical hyperplasia (CAH)
MMR-deficient Loss of ARID1A expression Overexpression of L1CAM Average number of methylated endometrial and ovarian cancer related genes out of total 7 Average number of methylated tumor suppressor genes out of total 24	30/31 (97%) 14/23 (61%) 3/22 (14%) 2.3 3.7	9/9 (100%) 9/9 (100%) 3/7 (43%) 3.7 3.8	14/14 (100%) 12/14 (86%) 2/13 (15%) 3.2 3.4	12/99 (12%) 0/22 (0%) N/A 0.70 2.4	5/12 (42%) 0/6 (0%) 0/4 (0%) 1.2 2.3	5/6 (83%) 1/4 (25%) 1/4 (25%) 2.3 3.0	33/38 (87%) 6/30 (20%) 1/28 (4%) 2.0 3.8

Abbreviations: N/A = not applicable. Proportions are based on cases that could be successfully analyzed.



Fig. 1. Molecular characteristics of synchronous carcinoma pairs from Lynch patients.

carcinoma comparisons (see footnote for Table 2). Interestingly, L1CAM was overexpressed in 43% (6/14) of tumors belonging to the 9 synchronous ovarian and endometrial carcinoma pairs, compared to 12% (3/25) of non-synchronous ovarian and endometrial carcinomas (p = 0.047). The latter observation further supports the interpretation of metastatic disease, since L1CAM is known to promote motility and invasion [31].

3.3. Targeted sequencing of synchronous carcinomas for somatic mutations

Sufficient DNA was available from 5 cases for targeted sequencing of 578 cancer-relevant genes as an additional means to assess clonal relatedness (Fig. 1, Table 3, Supplementary Table S3). Somatic mutation profiles indicated unequivocally shared origins for the paired tumors from three cases (LOC3, LOC18, and LOC13) and a tentatively shared origin for a fourth one (LOC16). The question of shared vs. independent origins remained unresolved in case LOC6 with ovarian clear cell carcinoma and ovarian borderline tumor sharing a single nonsynonymous lowfrequency mutation (in *CTNNB1*) predicted to be damaging (Table 3, Supplementary Table S3).

3.4. Comparison of endometrial hyperplasias and paired carcinomas for concordance

Molecular characteristics of endometrial hyperplasias preceding or coinciding with endometrial carcinoma are shown case by case in Fig. 2. Analogous data with ovarian carcinoma as the endpoint lesion are displayed in Fig. 3. Molecular alterations in endometrial hyperplasias were compared to those in the paired carcinomas and concordance/discordance assigned by the same criteria as for the carcinomacarcinoma comparisons above (Fig. 1). SH revealed a discordant pattern with synchronous or metachronous endometrial or ovarian carcinoma in all cases that could be evaluated (2/2, 100%, Figs. 2 and 3), arguing against premalignant potential of SH. CH and CAH were concordant with endometrial carcinoma as the endpoint lesion in 15 of 19 cases (79%) (CH 2/2 and CAH 13/17, Figs. 2 and 3), implying a cancer precursor role for complex hyperplasias without or with atypia. Interestingly, the proportion of concordant cases was comparable with ovarian carcinoma as the endpoint (9/11, 82%) (CH 0/1 and CAH 9/10, Fig. 3), suggesting that a developmental route from endometrial hyperplasia to ovarian carcinoma might also be possible. Cases LOC1 and LOC13 provide illustrative examples of a high molecular similarity of prior or concurrent CAH with ovarian carcinoma from the same cases. This observation together with the common origins of synchronous endometrial and ovarian carcinomas in LS as discussed above imply that

Table 2

Comparison of synchronous endometrial and ovarian carcinomas with the corresponding non-synchronous carcinomas from Lynch syndrome patients.

	Non-synchronous	Synchronous carcinomas $(n = 9)^a$		Non-synchronous		
	OvCa (<i>n</i> = 8)	OvCa	EnCa	EnCa ($n = 24$)		
MMR-deficient ARID1A negative	100% 100%	100% 89%	100% 86% (<i>n</i> = 7)	97% 59% (<i>n</i> = 17)		
L1CAM positive	13%	38% (<i>n</i> = 8)	50% (<i>n</i> = 6)	12% (<i>n</i> = 17)		
Average no. of methylation markers						
7 EnCa and OvCa related	3.38	3.44	2.56	2.13		
24 TSG	4.00	3.89	5.5 (<i>n</i> = 8)	2.74 (<i>n</i> = 23)		
Average Dm						
RSK4	0.64	0.71	0.71	0.61		
SPARC	0.62	0.71	0.63	0.53		
HOXA10	0.48	0.38	0.23	0.23		
HOXA9	0.62	0.67	0.54	0.43		
RASSF1	0.35	0.33	0.24 (<i>n</i> = 8)	0.28 (<i>n</i> = 23)		
CDH13	0.42	0.35	0.25 (<i>n</i> = 8)	0.31 (<i>n</i> = 23)		

Abbreviations: Dm, methylation dosage ratio; EnCa, endometrioid endometrial carcinoma; OvCa, ovarian carcinoma.

^a Includes one endocervical/ovarian carcinoma pair. All others are endometrioid endometrial/ovarian carcinoma pairs. If a result was not available from every sample, the actual number of samples is indicated in parentheses. Bolding indicates that a synchronous tumor exhibited the closest similarity to its pair rather than nonsynchronous tumors. The nine synchronous endometrial and ovarian carcinomas were compared relative to each other and to non-synchronous endometrial and ovarian carcinomas. Significant differences were detected only between synchronous OvCa and non-synchronous EnCa, as follows: Number of methylation markers (among 7 endometrial and ovarian cancer related genes), p = 0.042 by *t*-test; average Dm for *RSK4*, p = 0.036 by *t*-test; and average Dm for *HOXA9*, p = 0.045 by Mann-Whitney-U test.

Summary of Sc		u by synchronous Lync	II Cal CITIOTTIAS, Das	ed on deep sequencing of 578 cance	I-Ielevant genes.
	No. of somatic mu	utations, VarScan $p < 0$.01		
Case ID	Tumor 1	Tumor 2	Shared	Shared (relaxed criteria) ^a	Examples of shared somatic mutations predicted damaging ^t
LOC3	2915 (OvCC)	72 (EnCa)	2	N/A	TGFBR2 fs, JAK1 fs
LOC16	23 (OvE)	132 (EnCa)	0	4	ARID2 R1679Q, MAP2K2 E328K, SMARCB1 R350W
LOC18	38 (OvE)	1544 (EnCa)	10	N/A	PTEN K332*, PTEN fs
LOC13	65 (OvE)	78 (OvE)	41	N/A	PIK3CA R88Q, PTEN fs, ARID1A fs (two different)
LOC6	37 (OvCC)	41 (OvEB)	0	1	CTNNB1 R565H

Abbreviations: fs, frameshift; N/A, not applicable.

^a Somatic VarScan p < 0.05 in both tumors (indicated for cases without any shared mutations with p < 0.01).

^b A complete list of mutations is given in Supplementary Table S3.

the degree of molecular sharing between endometrial and ovarian tumorigenesis may be higher than appreciated before.

4. Discussion

The epidemiology of endometrial and ovarian cancer is intertwined, and several possible mechanisms, including hormonal and inflammation and immune system-related, may underlie this phenomenon [34]. LS provides an efficient tool to investigate the pathogenesis of endometrial and ovarian carcinoma for the following reasons: (i) the lifetime risks of these cancers are significantly elevated in LS compared to the average population [3,4], (ii) multiple lesions (both malignant and benign) in the same individuals are common [7,12], and (iii) lifelong surveillance against gynecological cancer results in consecutive specimens that are invaluable for research [35].

In LS, the clinically important issue of independent primary tumors vs. metastatic disease in the case of synchronous endometrial and ovarian cancers is unsettled, so far. We evaluated 13 synchronous carcinomas (9 endometrial plus ovarian carcinoma pairs, 3 cases with bilateral ovarian carcinomas, and one pair with endometrial and endocervical carcinoma) (Fig. 1) and our results indicate shared origins. Frequent L1CAM overexpression among the synchronous tumors was in agreement with the interpretation of metastatic disease. The available data are thus consistent with metastatic disease in synchronous cases from sporadic [14] and LS cases (this study); however, the direction of metastasis is unknown. Kelemen et al. [11] explored the patterns of molecular alterations (PTEN and MMR protein expression) in three groups of sporadic tumors: ovarian carcinomas synchronous with endometrial carcinoma, non-synchronous endometrial carcinomas, and nonsynchronous ovarian carcinomas. They found that ovarian carcinomas synchronous with endometrial carcinoma showed a greater similarity relative to non-synchronous endometrial carcinoma than nonsynchronous endometrioid ovarian carcinoma, possibly suggesting a metastatic spread from the endometrium to the ovary. In our LS series, synchronous ovarian carcinoma (or endometrial carcinoma) showed the closest molecular similarity to its synchronous pair, and a closer similarity to non-synchronous cancer of the same rather than the different organ (Table 2). Molecular similarity between tumors within each



Fig. 2. Molecular characteristics of endometrial hyperplasias occurring prior to or concurrently with endometrial carcinoma from Lynch syndrome patients.

Table 3



Fig. 3. Molecular characteristics of endometrial hyperplasias occurring prior to or concurrently with ovarian carcinoma (and synchronous endometrial carcinoma) from Lynch syndrome patients.

pair may suggest that metastasis occurs soon after the first tumor has arisen. No conclusions about the direction of metastasis can be drawn.

In the sporadic setting, L1CAM overexpression has been found to be an adverse prognostic sign [31]. Among stage I endometrioid endometrial cancers, which are usually associated with excellent prognosis, L1CAM overexpression identifies a subgroup with significantly poorer disease-free and overall survival [32]. Among ovarian carcinomas, L1CAM overexpression signals poor outcome of endometrioid, but not clear cell type [33]. In ovarian and endometrial carcinomas from our LS patients, L1CAM overexpression was significantly more common in synchronous (43%) than non-synchronous cases (12%). Patients with synchronous carcinomas exhibited excellent survival (the crude 10year survival was 83%) with no apparent association with L1CAM expression (data not shown). Synchronous endometrial and ovarian carcinomas from sporadic cases, too, are associated with indolent course, which is unexpected of a metastatic disease. An isolated metastatic disease distinct from the usual progressive metastatic disease was proposed as a possible explanation [13,14].

The accumulating evidence suggests that endometrioid and clear cell ovarian carcinomas arise from endometrial epithelial cells via atypical endometriosis and borderline tumors [36]. It remains to be resolved if mutations in endometriosis are critical or alternatively, if mutations in eutopic endometrium might first cause predisposition to endometriosis that subsequently develops into malignancy [18]. Our study reveals that endometrial hyperplasias show a comparable degree of concordance relative to endometrial carcinoma vs. ovarian carcinoma as the endpoint lesions (Figs. 2 and 3). Based on our findings, a possible role for endometrial hyperplasias in ovarian tumorigenesis cannot be excluded and additional research is warranted. In line with this notion, our Lynch series includes two cases with CAH treated with hysterectomy without prophylactic salpingo-oophorectomy and both developed ovarian carcinoma (LOC1 was diagnosed with OvE 3 years later and LOC22 with bilateral OvCC 7 years later; Fig. 3).

Compared to many existing studies, the longitudinal sample cohort from over two decades of gynecological surveillance is a major advantage of our investigation. The availability of synchronous and nonsynchronous endometrial and ovarian carcinomas, as well as consecutive endometrial specimens preceding the malignant stage, formed an excellent basis to dissect the relationship between endometrial and ovarian tumorigenesis. The inclusion of both genetic and epigenetic markers in molecular evaluations can also be considered as a strength. On the other hand, our investigation has some important limitations. The amount and/or quality of archival tissue specimens was sometimes suboptimal for a successful completion of all intended analyses. Moreover, our deep sequencing experiments relied on a gene panel instead of whole exomes; nevertheless, the complete coding regions of 578 established cancer driver genes (e.g., *ARID1A, PTEN*, and *PIK3CA* relevant for endometrioid endometrial and non-serous ovarian carcinomas) were covered.

In conclusion, our genetic and epigenetic analyses indicate shared origins for synchronous endometrial and ovarian carcinomas in LS. Moreover, endometrial hyperplasias detected in a long-term surveillance program exhibit close molecular similarity to endometrial carcinomas and likewise ovarian carcinomas as the endpoint lesions, suggesting early convergence of endometrial and ovarian tumorigenesis. In MMR gene mutation carriers, surveillance for endometrial cancer by gynecological examination, transvaginal ultrasound and aspiration biopsy is recommended starting from age 35-40 years with the primary aim to detect premalignant lesions (endometrial hyperplasia) or earlystage endometrial carcinoma [35,37]. Furthermore, prophylactic hysterectomy and bilateral oophorectomy which prevent the development of endometrial and ovarian cancer, is recommended for mutation carriers who have completed their families [35,37]. The multilevel ties we observed between endometrial and ovarian tumorigenesis emphasize that whenever an endometrial lesion (CH, CAH, or endometrial cancer) is detected, the possibility of ovarian involvement should be kept in mind and vice versa.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2018.04.566.

Acknowledgments

We are grateful to the patients and responsible clinical experts for participation and Saila Saarinen for expert technical assistance. This work was supported by Jane and Aatos Erkko Foundation (to P.P. and J.-P.M.), the Academy of Finland (grant no. 294643, to P.P.), the Finnish Cancer Organizations (to P.P. and J.-P.M.), the Sigrid Juselius Foundation (to P.P.), the HiLIFE Fellows 2017–2020 (to P.P.), the Integrative Life Science Doctoral Program ILS (to A.N.), and the K Albin Johanssons stiffelse (to A.N.).

Disclosure statement

The authors report no conflict of interest.

References

- [1] Society® AC, Cancer Facts & Figures, 2018 (2018).
- [2] B.A. Thompson, A.B. Spurdle, J.P. Plazzer, M.S. Greenblatt, K. Akagi, F. Al-Mulla, et al., Application of a 5-tiered scheme for standardized classification of 2,360 unique mismatch repair gene variants in the InSiGHT locus-specific database, Nat. Genet. 46 (2014) 107–115.
- [3] V. Bonadona, B. Bonaiti, S. Olschwang, S. Grandjouan, L. Huiart, M. Longy, et al., Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome, JAMA 305 (2011) 2304–2310.

- [4] P. Moller, T. Seppala, I. Bernstein, E. Holinski-Feder, P. Sala, D.G. Evans, et al., Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database, Gut 66 (2017) 464–472.
- [5] K.H. Lu, J.O. Schorge, K.J. Rodabaugh, M.S. Daniels, C.C. Sun, P.T. Soliman, et al., Prospective determination of prevalence of lynch syndrome in young women with endometrial cancer, J. Clin. Oncol. 25 (2007) 5158–5164.
- [6] S. Malander, E. Rambech, U. Kristoffersson, B. Halvarsson, M. Ridderheim, A. Borg, et al., The contribution of the hereditary nonpolyposis colorectal cancer syndrome to the development of ovarian cancer, Gynecol. Oncol. 101 (2006) 238–243.
- [7] L. Rossi, M.A. Le Frere-Belda, P. Laurent-Puig, B. Buecher, A. De Pauw, D. Stoppa-Lyonnet, et al., Clinicopathologic characteristics of endometrial cancer in lynch syndrome: a French multicenter study, Int. J. Gynecol. Cancer 27 (2017) 953–960.
- [8] E.M. Grindedal, L. Renkonen-Sinisalo, H. Vasen, G. Evans, P. Sala, I. Blanco, et al., Survival in women with MMR mutations and ovarian cancer: a multicentre study in Lynch syndrome kindreds, J. Med. Genet. 47 (2010) 99–102.
- [9] J.M. Helder-Woolderink, E.A. Blok, H.F. Vasen, H. Hollema, M.J. Mourits, G.H. De Bock, Ovarian cancer in Lynch syndrome; a systematic review, Eur. J. Cancer 55 (2016) 65–73.
- [10] R.J. Kurman, M. Shih le, Pathogenesis of ovarian cancer: lessons from morphology and molecular biology and their clinical implications, Int. J. Gynecol. Pathol. 27 (2008) 151–160.
- [11] L.E. Kelemen, P.F. Rambau, J.M. Koziak, H. Steed, M. Kobel, Synchronous endometrial and ovarian carcinomas: predictors of risk and associations with survival and tumor expression profiles, Cancer Causes Control 28 (2017) 447–457.
- [12] N.A. Ryan, D.G. Evans, K. Green, E.J. Crosbie, Pathological features and clinical behavior of Lynch syndrome-associated ovarian cancer, Gynecol. Oncol. 144 (2017) 491–495.
- [13] M.S. Anglesio, Y.K. Wang, M. Maassen, H.M. Horlings, A. Bashashati, J. Senz, et al., Synchronous endometrial and ovarian carcinomas: evidence of clonality, J. Natl. Cancer Inst. 108 (2016), djv428.
- [14] A.M. Schultheis, C.K. Ng, M.R. De Filippo, S. Piscuoglio, G.S. Macedo, S. Gatius, et al., Massively parallel sequencing-based clonality analysis of synchronous endometrioid endometrial and ovarian carcinomas, J. Natl. Cancer Inst. 108 (2016), djv427.
- [15] S.R. de Rijk, M.E. Steenbergen, T.E. Nieboer, S.F. Coppus, Atypical endometrial polyps and concurrent endometrial cancer: a systematic review, Obstet. Gynecol. 128 (2016) 519–525.
- [16] J. Prat, A. Gallardo, M. Cuatrecasas, L. Catasus, Endometrial carcinoma: pathology and genetics, Pathology 39 (2007) 72–87.
- [17] M.J. Mingels, R. Masadah, Y.P. Geels, I. Otte-Holler, I.M. de Kievit, J.A. van der Laak, et al., High prevalence of atypical hyperplasia in the endometrium of patients with epithelial ovarian cancer, Am. J. Clin. Pathol. 142 (2014) 213–221.
- [18] I. Gounaris, D.S. Charnock-Jones, J.D. Brenton, Ovarian clear cell carcinoma-bad endometriosis or bad endometrium? J. Pathol. 225 (2011) 157–160.
- [19] R.E. Scully, H.E. Poulsen, Histological Typing of Female Genital Tract Tumours, 2nd ed. Springer-Verlag, Berlin; New York, 1994.
- [20] S.G.K.R. Silverberg, F. Nogales, G.L. Mutter, R.A. Kubik-Huch, F.A. Tavassoli, Epithelial tumours and related lesions, in: F.A.D.P. Tavassoli (Ed.), Pathology and Genetics of Tumours of the Breast and Female Genital Organs World Health Organization Classification of Tumours, IARC Press, Lyon (France) 2003, pp. 221–232.

- [21] J. Isola, S. DeVries, L. Chu, S. Ghazvini, F. Waldman, Analysis of changes in DNA sequence copy number by comparative genomic hybridization in archival paraffinembedded tumor samples, Am. J. Pathol. 145 (1994) 1301–1308.
- [22] A. Loukola, K. Eklin, P. Laiho, R. Salovaara, P. Kristo, H. Jarvinen, et al., Microsatellite marker analysis in screening for hereditary nonpolyposis colorectal cancer (HNPCC), Cancer Res. 61 (2001) 4545–4549.
- [23] A. Niskakoski, S. Kaur, L. Renkonen-Sinisalo, H. Lassus, H.J. Jarvinen, J.P. Mecklin, et al., Distinct molecular profiles in Lynch syndrome-associated and sporadic ovarian carcinomas, Int. J. Cancer 133 (2013) 2596–2608.
- [24] A. Niskakoski, S. Kaur, S. Staff, L. Renkonen-Sinisalo, H. Lassus, H.J. Jarvinen, et al., Epigenetic analysis of sporadic and Lynch-associated ovarian cancers reveals histology-specific patterns of DNA methylation, Epigenetics 9 (2014) 1577–1587.
- [25] A.H. Gylling, T.T. Nieminen, W.M. Abdel-Rahman, K. Nuorva, M. Juhola, E.I. Joensuu, et al., Differential cancer predisposition in Lynch syndrome: insights from molecular analysis of brain and urinary tract tumors, Carcinogenesis 29 (2008) 1351–1359.
- [26] N. Porkka, S. Valo, T.T. Nieminen, A. Olkinuora, S. Maki-Nevala, S. Eldfors, et al., Sequencing of Lynch syndrome tumors reveals the importance of epigenetic alterations, Oncotarget 8 (2017) 108020–108030.
- [27] A.M. Sulonen, P. Ellonen, H. Almusa, M. Lepisto, S. Eldfors, S. Hannula, et al., Comparison of solution-based exome capture methods for next generation sequencing, Genome Biol. 12 (2011) R94.
- [28] D.C. Koboldt, Q. Zhang, D.E. Larson, D. Shen, M.D. McLellan, L. Lin, et al., VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing, Genome Res. 22 (2012) 568–576.
- [29] L. Renkonen-Sinisalo, R. Butzow, A. Leminen, P. Lehtovirta, J.P. Mecklin, H.J. Jarvinen, Surveillance for endometrial cancer in hereditary nonpolyposis colorectal cancer syndrome, Int. J. Cancer 120 (4) (2007) 821.
- [30] A. Niskakoski, A. Pasanen, H. Lassus, L. Renkonen-Sinisalo, S. Kaur, J.-P. Mecklin, R. Bützow, P. Peltomäki, Molecular changes preceding endometrial and ovarian cancer: a study of consecutive endometrial specimens from Lynch syndrome surveillance, Mod. Pathol. (2018) https://doi.org/10.1038/s41379-018-0044-4 (In Press).
- [31] P. Altevogt, K. Doberstein, M. Fogel, L1CAM in human cancer, Int. J. Cancer 138 (2016) 1565–1576.
- [32] A.G. Zeimet, D. Reimer, M. Huszar, B. Winterhoff, U. Puistola, S.A. Azim, et al., L1CAM in early-stage type I endometrial cancer: results of a large multicenter evaluation, J. Natl. Cancer Inst. 105 (2013) 1142–1150.
- [33] P. Soovares, A. Pasanen, R. Butzow, H. Lassus, L1CAM expression associates with poor outcome in endometrioid, but not in clear cell ovarian carcinoma, Gynecol. Oncol. 146 (2017) 615–622.
- [34] D.W. Cramer, The epidemiology of endometrial and ovarian cancer, Hematol. Oncol. Clin. North Am. 26 (2012) 1–12.
- [35] K.H. Lu, M. Daniels, Endometrial and ovarian cancer in women with Lynch syndrome: update in screening and prevention, Familial Cancer 12 (2013) 273–277.
- [36] A.N. Karnezis, K.R. Cho, C.B. Gilks, C.L. Pearce, D.G. Huntsman, The disparate origins of ovarian cancers: pathogenesis and prevention strategies, Nat. Rev. Cancer 17 (2017) 65–74.
- [37] H.F. Vasen, I. Blanco, K. Aktan-Collan, J.P. Gopie, A. Alonso, S. Aretz, et al., Revised guidelines for the clinical management of Lynch syndrome (HNPCC): recommendations by a group of European experts, Gut 62 (2013) 812–823.