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

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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.

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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.

1. Introduction

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

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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.mrc-holland.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

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