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Ovarian Cancer at Young Age;

the contribution of mismatch-repair defects in a population-based series of epithelial ovarian cancer before age 40

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Abstract

At least one out of 10 patients with ovarian cancer is estimated to develop their tumor because of heredity with the breast and ovarian cancer syndrome due to mutations in the *BRCA1* and *BRCA2* genes and hereditary nonpolyposis colorectal cancer (HNPCC) being the major genetic causes. Cancer at young age is a hallmark of heredity and ovarian cancers associated with HNPCC have been demonstrated to develop at a particularly early age. We utilized the Swedish Cancer Registry to identify a population-based series of 98 invasive epithelial ovarian cancers that developed ≤ 40 years of age. Mucinous and endometrioid cancers were overrepresented and were diagnosed in 27% and 16% of the tumors respectively.

Immunostaining using antibodies against MLH1, PMS2, MSH2, and MSH6 was used to assess the mismatch-repair (MMR) status and revealed loss of expression of MLH1/PMS2 in two cases, loss of MSH2/MSH6 in one case, and loss of MSH6 only in three tumors. A MSI-high phenotype was verified in 5 of the 6 tumors. Based on identified mutations and family history of cancer, several of these individuals are likely to be affected by HNPCC. We conclude that although the causes of the vast majority of epithelial ovarian cancer at young age are unknown, HNPCC should be considered because of the high risk of metachronous colorectal cancer in the individual and the possibility of preventing additional cancers in the family through control programmes.

Introduction

Ovarian cancer is a major cause of death from gynecologic cancer and heredity is estimated to cause at least 10% of the cases ^(1,2). Although ovarian cancer has most commonly been associated with hereditary breast and ovarian cancer due to mutations in the *BRCA*-genes, ovarian cancer also occurs within the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome, and as site specific ovarian cancer with a yet unknown genetic background ^(2,3). Characterization of familial predisposition to ovarian cancer and the underlying genetic causes hereof is important since risks may apply also for other tumor types e.g. breast cancer, colorectal cancer, and endometrial cancer for which preventive measures may be beneficial. Although control programmes have not proven effective for ovarian cancer, prophylactic salpingo-oophorectomy effectively reduces cancer risk in women with a hereditary predisposition for ovarian cancer ⁽⁴⁻⁶⁾.

HNPCC, or Lynch syndrome, is an autosomal dominant cancer syndrome in which mutation carriers have increased life-time risks of several cancer types, with the highest risks for colorectal cancer (80%), endometrial cancer (40-60%), and ovarian cancer (12%) ^(7,8). Increased risks also apply to rare tumor types e.g. cancer of the small intestine, upper urinary tract cancer, gastric cancer, brain tumors, and sebaceous skin tumors. HNPCC is caused by germline mismatch repair (MMR) gene mutations most commonly affecting *MLH1*, *MSH2*, and *MSH6* with more than 500 mutations in these genes identified worldwide ⁽⁹⁾. The underlying genetic defect causes widespread microsatellite instability (MSI) in the tumors and this phenomenon is, together with an immunohistochemical loss of expression of the affected MMR protein, utilized in the diagnosis of HNPCC ⁽¹⁰⁻¹²⁾.

Approximately 2% of ovarian cancer has been estimated to be caused by HNPCC and these tumors represent epithelial ovarian cancers that often develop at younger age, mean 41-49 years, compared to the sporadic cases with a mean age of 60-65 years⁽¹³⁻¹⁵⁾. In order to determine the frequency of defective MMR and the contribution of the various MMR genes in the development of ovarian at young age, we characterized the expression of MLH1, PMS2, MSH2, and MSH6 in a population-based series of 98 women who developed epithelial ovarian cancer at young (≤ 40 years) age.

Patients and Methods

Collection of materials

Ethical approval for the study was granted by the ethics committee at Lund University. The regional part of the National Swedish Cancer Registry was used to identify all ovarian malignancies diagnosed ≤ 40 years of age during the time period January 1970 through December 2000. All pathology reports were retrieved and nonepithelial (mainly germ cell tumors) and borderline tumors were excluded. Hereafter 130 patients remained, from whom paraffin-embedded tumor tissue could be obtained in 98 cases. All histopathologic slides were reviewed by a gynaecologic pathologist (A.M.) to confirm the diagnosis of an invasive epithelial malignancy and the histopathologic subtype. The mean age was 36 (range 21-40) years and the serous/seropapillary tumors and mucinous tumors represented the largest histopathological subsets (table 1).

Second primary malignancies among the 98 women were identified through the National Cancer Registry and family histories of cancer were collected from clinical files and the cancer cases were confirmed in the Cancer Registry.

Immunohistochemistry

The MMR proteins MLH1, MSH2, MSH6 and PMS2 were immunohistochemically examined on fresh 4- μ m sections from paraffin-embedded tumor blocks. The sections were mounted on DAKO ChemMate Capillary Gap Microscope Slides (DAKO A/S Glostrup, Denmark), dried at room temperature overnight, followed by 1-2 hour incubation at 60°C. Xylol was used for deparaffinisation and for rehydration the slides were run through a series of descending alcohol concentrations. Antigen retrieval was achieved by microwave treatment in 10 mM Tris, 1mM EDTA, pH 9, at 800 W for 8 minutes, followed by 15 minutes at 300 W.

Afterwards the slides were left to cool in the Tris-EDTA solution for 20 minutes.

Immunohistochemical staining was performed in an automated immunostainer (TechmateTM 500 Plus, DAKO A/S Glostrup, Denmark) according to the manufacturers' instructions. The procedure included incubation in room temperature for 25 minutes with primary antibodies.

These were mouse-anti-human, monoclonal IgG: MLH1 (clone G 168-15, dilution 1:200, PharMingen, San Diego, CA, USA), MSH2 (clone FE-11, dilution 1:100, Oncogene Research Products, San Diego, CA, USA), MSH6 (clone 44, dilution 1:1000, BD Transduction Laboratories, Lexington, KY, USA) and PMS2 (clone A16-4, dilution 1:500, BD PharMingen, San Diego, CA, USA). The ChemMate EnVisionTM Detection Kit was used, and an extra enhancing step with Rabbit Anti Mouse immunoglobulins (dilution 1:400, DAKO A/S Glostrup, Denmark) was performed after incubation with primary antibodies.

Diaminobenzidine was used as a chromogen. The slides were counterstained with hematoxylin, dehydrated in decreasing concentrations of alcohol and mounted. Three of the authors (S.M., K.D. and M.N.) independently evaluated the results of the immunohistochemistry. Loss of MMR proteins was defined as absence of nuclear staining in the presence of a retained staining within the stroma components of the tumor.

Immunohistochemical staining of tumor infiltrating lymphocytes, tumor stroma and/or normal surrounding tissue served as an internal positive control.

Microsatellite instability (MSI)

DNA was extracted from three 10- μ m sections of paraffin-embedded tissue through incubation in EDTA-Tris-buffer with Proteinase K at 65°C for at least 2 hours, followed by boiling, centrifugation and removal of the aqueous phase, which was stored at 4°C. The microsatellite status was determined using the markers BAT25, BAT26, BAT34C4 and BAT40, all of which are among the markers recommended by the National Cancer Institute (NCI) ⁽¹⁶⁾. The markers were fluorescently labeled with NED™, 6-FAM™, and HEX™ and the primer sequences and PCR conditions are available from the authors upon request. The PCR products were mixed with 12 μ L deionized formamide (Hi-Di Formamide, Applied Biosystems, Foster City, CA, USA) and 0.5 μ L ROX™ 500 Size Standard (Applied Biosystems, Foster City, CA, USA). The DNA was denatured at 95°C for 2 minutes, cooled on ice and separated in Performance Optimized Polymer-4 (POP-4™) on the ABI PRISM™ 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). MSI was defined as the presence of extra peaks and were, according to the NCI guidelines, considered MSI-low if this applied to one marker and MSI-high if at least two markers were affected.

Results

Among the ovarian cancers that developed in this population-based series 58/98 (59%) were early stage (table 1). Tumor histology was serous in 45% of the tumors, followed by mucinous in 27%, and endometrioid in 16% (table 1). Metachronous cancers developed in 16 (16%) individuals and included 6 cervical cancers, 3 lung cancer, 2 breast cancers, 2 urinary bladder tumors, one malignant melanoma, one pancreatic cancer, and one acute myeloid

leukaemia. Of these, 6 malignancies developed at a mean of 5 (range 3-11) years before the ovarian cancer diagnosis and 10 developed at a mean of 15 (5-25) years after the ovarian cancer. Immunostaining using antibodies against MLH1, PMS2, MSH2, and MSH6 revealed concomitant loss of MLH1/PMS2 in two tumors (figure 1), concomitant loss of MSH2/MSH6 in one tumor and loss of MSH6 only in three tumors (table 2). MSI analysis was performed on the 6 tumors that showed loss of staining in order to confirm the MSI status and revealed a MSI-high phenotype with instability for the markers BAT25, BAT26 and BAT 40, but not for BAT34C4 in 5 tumors, whereas the remaining tumor (with loss of MSH6) showed a microsatellite stable phenotype (table 2).

Discussion

Although tumor development at young age is a hallmark of heredity, differences in the contribution of heredity and the age of onset seem to apply for different tumor types as well as for the different hereditary syndromes. In colorectal cancer, 10-20% of the tumors that develop before age 45 are estimated to be caused by HNPCC and an additional fraction is probably caused by other types of heredity⁽¹⁷⁾. Studies that have assessed the contribution of the breast-ovarian cancer syndrome to the development of breast cancer before age 45 have concluded that 5-10% of the cases are caused by mutations in *BRCA1* and *BRCA2*⁽¹⁸⁻²⁰⁾. Hereditary ovarian cancer has primarily been associated with *BRCA* mutations with 20-40% life-time risk of ovarian cancer for mutation carriers and tumor development at a mean age of 50-55 years^(21,22). Assessment of *BRCA* mutations among women diagnosed before age 30 have, however, not identified *BRCA* gene mutations, but rather suggested involvement of HNPCC in young females with invasive epithelial ovarian cancer⁽²³⁾. HNPCC has been estimated to contribute to about 2% of ovarian cancer, thus to an equal overall proportion as

to colorectal cancer and endometrial cancer, but is associated with early tumor development with most affected individuals being in their forties ^(13-15, 23).

Overall, a MSI-high phenotype has been identified in 18% of ovarian cancer ^(16,24-30).

However, only occasional tumors are associated with HNPCC, and about half of the tumors have shown somatic hypermethylation of the *MLH1* promotor, which suggests that other genetic causes underly the remaining MSI tumors ⁽²⁴⁾. We identified MMR-defects in 6 of the 98 tumors studied. Only one case (patient 37) had no family history of cancer and developed a clear cell cancer with concomitant loss of MLH1/PMS2, which is likely to indicate somatic MMR gene inactivation. This is supported also by the identification of a *BRAF* V599E mutation in this tumor (data not shown). The remaining tumors are likely to be associated with HNPCC based on loss of MSH2, family history of cancer or identification of a disease-causing germline mutation (table 2). Only 2 women (both of whom had tumors with retained MMR expression) developed metachronous breast cancer at ages 43 and 58 years, respectively, which indicates that the breast-ovarian cancer syndrome has a minor, if any, importance for ovarian cancer development at young age. This is also in line with the findings by Stratton et al. who did not identify any BRCA cases among 101 individuals with early-onset ovarian cancer, whereas HNPCC-causing mutations were identified in 2% of the women ⁽³¹⁾. This estimated may represent an underestimate since large intragenic deletions and mutations in *MSH6* were not accounted for. In our series, three tumors showed loss of MSH6 only, which may suggest an underlying mutation in this gene. *MSH6* has been shown to confer a lower risk of colorectal cancer and a particularly high risk of endometrial cancer ⁽³²⁾. The identification of HNPCC cases among women with ovarian cancer at young age implicates that the family history of cancer should include also other types of HNPCC-related cancers, most commonly colorectal cancer and endometrial, which is important since

prophylactic measures and control programmes for these tumor types have proven effective^(5,33). However, the vast majority of ovarian cancers, also at young age, develops because of unknown mechanisms and can not be linked to the currently identified genetic syndromes.

Serous and seropapillary tumors constitute about 50% of ovarian cancer, whereas endometrioid, clear cell, and mucinous tumors each constitute about 10% of the tumors⁽³⁴⁾. In our study mucinous and endometrioid tumors were overrepresented and occurred in 27% and 16%, respectively (table 1), which is in line with the high proportion of mucinous tumors identified by Stratton et al.⁽³¹⁾. Among the MMR defective ovarian cancers identified herein, 3 were endometrioid, 2 were clear cell cancers, and one was a mucinous ovarian cancer. Both endometrial cancers and clear cell cancers represent rare subtypes that show MMR defects in a high (14-37%) fraction of the tumors and these histopathological types have also been linked to HNPCC^(14,26-29).

Conclusion

In summary, we identified a large fraction of mucinous and endometrioid cancers in this population-based cohort of young women with epithelial ovarian cancer. Defective MMR was identified in 6% of the tumors and suggests that HNPCC has a larger impact on ovarian cancer development in young women than the more frequently recognized hereditary breast and ovarian cancer syndrome. Identification of HNPCC-families is important since control programmes may effectively prevent additional cases of the more common colorectal and endometrial cancers. However, even among young women 9 out of 10 tumors develop because of unknown causes, which suggests that other mechanisms need to be studied to reveal if the high frequency of mucinous and endometrioid ovarian cancers may reflect distinct tumorigenic pathways.

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Legend to figure:

Fig. 1 MMR protein immunostainings (patient 37) showing retained nuclear staining for MSH2 and MSH6 (upper row) and loss of nuclear staining in the tumor cells with retained staining in the stromal components for MLH1 and PMS2 (lower row). Concomitant losses reflect functional interactions between these MMR proteins.

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