No BRCA1 germline mutation in a family with uterine papillary serous carcinoma: A case report

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Summary

The purpose of the study was to examine BRCA1 germline mutation and its relationship to BRCA1 expression in two patients, a mother and a daughter, both diagnosed with uterine papillary serous carcinoma (UPSC). DNA was screened for BRCA1 and BRCA2 germline mutations common in the Jewish population (185delAG, 5382insC, and 6174delT) by PCR-based assay and with a protein truncation test (PTT) to detect mutation in exon 11 of BRCA1 and exons 10 and 11 of BRCA2. BRCA1 expression in fixed tumor tissues was assessed by immunocytochemistry (IHC). No germline mutation in either BRCA1 or BRCA2 gene was found in the two patients. Both samples showed reduced levels of BRCA1 expression. Taken together, these results suggest that undetected or unscreened for germline mutation may be associated with occurrence of this rare tumor type in two members of the same family. Alternatively, an epigenetic mechanism such as BRCA1promoter hypermethylation may be responsible for reduced expression of BRCA1 in the absence of DNA mutations.

Key words: Uterine papillary serous carcinoma; BRCA1; BRCA2; Immunocytochemistry.

Introduction

Uterine papillary serous carcinoma (UPSC) is a distinct histologic subtype of endometrial carcinoma accounting for 10% of all endometrial cancers [1]. Compared with more frequent endometrioid adenocarcinoma, UPSC is more aggressive, has propensity for early spread to the ovaries and the peritoneal cavity, and has more extensive lymphatic involvement and recurrence pattern, particularly in the upper abdomen. Therefore, it has been suggested that UPSC resembles ovarian seropapillary carcinoma, both histologically and clinically [2].

While the genetic background for UPSC is largely unknown, germline mutations in BRCA1 and BRCA2 genes are genetic hallmarks of hereditary ovarian and breast-ovarian cancer syndromes. Although mutation analyses have shown over 100 mutations in each gene, three mutations are recurrent in the Ashkenazi Jewish women, and their combined frequency in this population is 2.5%. The three founder mutations: 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 cover almost all cases of familial disease in this population. Although there is no reported excess of endometrial carcinoma in women with BRCA1 and BRCA2 mutations, in 1999 Hornereich et al. reported a family with BRCA1 5383insC mutation in which one sister developed UPSC, while the other sister developed seropapillary carcinoma of the ovary. This suggested a genetic link between BRCA1 mutation and UPSC, as well as, oncogenic relation of ovarian and uterine seropapillary carcinomas [3]. Following studies however, found a lower incidence of BRCA1 mutations in Jewish women with UPSC who had a history of breast or ovarian cancer [4] and no mutations at all in a large series of patients with sporadic UPSC [5].

We present a family in which a mother and one daughter were diagnosed with UPSC, and present the results of targeted BRCA1 and BRCA2 mutation analysis and its relationship to BRCA1 expression pattern in two of the patients available for the analysis.

Case Report

Patient 1 is a 73-year-old woman, G3P3003, menopausal since her late forties, who never received hormone replacement therapy. She reported a 6-month history of vaginal spotting and an endometrial biopsy was done which showed endometrial cancer. She underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH-BSO), bilateral pelvic lymph node dissection, and pelvic washings. The final pathology showed papillary serous carcinoma of the endometrium with less than 50% myometrial invasion and with positive vascular invasion, but without endocervical, ovarian, or lymphatic involvement. Thus, stage IB disease was documented. The patient received six courses of adjuvant topotecan (Yale Protocol) and was without evidence of disease for 24 months, with a recent recurrence documented.

Immediately preceding the diagnosis in this patient, one of her three daughters, a 46-year-old nulligravid woman presented with a 2-month history of abnormal bleeding. Endometrial curettings showed a moderately differentiated endometrioid adenocarcinoma, with high nuclear features suspicious for serous differentiation. She underwent surgical staging, including TAH, BSO, omentectomy, and pelvic and paraaortic lymph-node sampling. Final pathology showed stage IIIA moderately differentiated endometrioid carcinoma with focal seropapillary features. All omental and nodal tissue was negative, however she did have serosal involvement, extensive lymphovascular space involvement and metastases to the right fallopian tube. She completed six cycles of adjuvant single agent topotecan chemotherapy and has no evidence of the disease 23 months after the surgery.

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The patients are of Jewish origin. Their family history includes two other sister without the disease. There is no history of ovarian cancer in the family, however a distant cousin was diagnosed with breast cancer in her mid-thirties and she is alive without evidence of the disease 17 years after the diagnosis. Both patients gave informed consent for BRCA1 and BRCA2 germline mutations. The remaining two sisters were not available for testing.

Methods

Mutation Screening

DNA was extracted from peripheral blood lymphocytes by use of DNA Mini Blood Extraction Kit (Quiagen). Exons 2 and 20 of the BRCA1 gene (for the 185delAG and 5382insC mutation) and exon 11 of the BRCA2 gene (for the 6174delT mutation) were amplified by polymerase chain reaction (PCR) using primer sequences described previously [6]. The PCR products were checked for fragment size on 7% polyacrylamide (PAGE) gels and subsequently subjected to dye terminator sequencing using performance optimized polymer 6 on the ABI PRISM™ 310 Genetic Analyzer (PE Applied Biosystems). The majority of mutations in BRCA1 and BRCA2 are frame-shift and nonsense mutations resulting in truncation or absence of the protein product; further, a major number of these mutations are present in large exon 11 of both the genes. Thus, the protein truncation test (PTT), a rapid and sensitive method to detect frameshift and nonsense mutation [7] was used in screening BRCA1 exon 11 and BRCA2 exons 10 and 11 to look for mutations other than Jewish founder mutations. DNA was amplified in partly overlapping fragments, transcribed and translated into proteins using TNT T7 Quick Coupled Transcription/Translation System Promega, and the proteins separated on SDS-PAGE gels. Dried gels were exposed overnight to Biomax X-ray films.

BRCA1 IHC Analysis

Mouse monoclonal antibody against human BRCA1 protein (Ab-1, IgG2a) generated from amino acid sequence 1-304 of BRCA1 was obtained from Oncogene Research Products (Cambridge, MA). IHC analysis was performed on formalin-fixed and paraffin-embedded sections using the streptavidin-biotin-perohidase methodology. BRCA1 antigen was unmasked with the heat-mediated antigen retrieval method [8]. The tissue sections were incubated overnight at 4°C with the monoclonal antibody at a dilution 1:100 10ug/ml) in PBS with 1% bovine serum albumin. The LSAB+ reagents (DACO, Carpenteria, CA) were used as recommended. Visualization was carried out with diaminobenzidine tetrahydrochloride as chromogen (BioGenex). Quantitative assessment of IHC results was based on distinct nuclear staining. Moderate or strong nuclear staining of control specimens of ovarian follicles was considered as positive. Percentage of positively stained cells was estimated after counting a total of 200 epithelial tumor cells from each case.

Results

There was no germline mutation of BRCA1 or BRCA2 found in either patient when tested for the 3-founder mutations or with PTT testing of exon 11 of BRCA1 and exons 10 and 11 of BRCA2.

IHC studies showed that BRCA1 expression in normal ovarian tissue was localized in granulosa and theca internal cell nuclei of developing follicles; the pattern of follicular staining was uniform and of moderate intensity. BRCA1 immunoreactivity was reduced in both tumor samples; with 85/500 mildly positive nuclei as compared with 140/500 in the control tissue in tumor 1, and 43/500 mildly positive cells in tumor 2 compared with 217/500 in the control tissue.

Discussion

Several lines of evidence suggest similar origin of seropapillary ovarian carcinoma and UPSC. Both tumors have aggressive biological behavior and similar patterns of spread and recurrence, and propensity for involvement of peritoneal surfaces. The spectrum of somatic mutations involved in UPSC is different form endometrioid adenocarcinoma, but similar to ovarian carcinoma, e.g., p53 mutations are present in 80-90% of UPSC and in more than 50% of ovarian carcinoma [9]. Furthermore, recently Hornreich *et al.* reported an association between BRCA1 mutation and uterine serous papillary carcinoma [3]. Lavie *et al.* reported the presence of 185delAG and 5382insC germline mutations respectively, in two Ashkenazi Jewish women diagnosed with serous papillary uterine carcinoma [4]. Both carriers also had family histories suggestive of familial breast or ovarian cancer.

Based on these similarities it has been postulated that UPSC may constitute a manifestation of abnormal BRCA1 expression [3]. However, in the largest study to date Goshen et al. found no germline mutations of BRCA1 or BRCA2 in any of the 56 non-Jewish patients with UPSC [5].

We failed to detect BRCA1 or BRCA2 mutation in a Jewish family in which both mother and daughter developed UPSC. We assessed the presence of the 3-founder mutation by PCR-based assay and PTT to detect mutations in exondel 11 of BRCA1 and in exons 10 and 11 of BRCA2, recently reported to be frequently involved in sporadic ovarian cancer [10]. We believe that the assays we employed would detect more than 90% of all BRCA1 and BRCA2 mutations.

However, we did find reduced BRCA1 expression in tumor tissue from both patient with UPSC. This parallels similar results of reduced BRCA1 expression in 38 unselected sporadic ovarian carcinomas [11]. Taken together, our results of lack of BRCA1 and BRCA2 mutations and reduced BRCA1 expression can be explained in two ways. It is possible that the familial aggregation of UPSC is due to BRCA1 or BRCA2 mutations not detectable with the assays we used. Alternatively, lack of BRCA1 mutations and lower BRCA1 expression may be due to an epigenetic mechanism such as hypermethylation of the BRCA1 promoter region. Methylation of BRCA1promoter has been associated with absent BRCA1 protein expression in sporadic ovarian cancer [12]. Promoter hypermethylation may therefore be an alternative to mutations in causing the inactivation of the BRCA1 in UPSC.

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