Evidence for a Multifocal Origin of Papillary Serous Carcinoma of the Peritoneum


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Abstract

Histopathological evidence suggests that papillary serous carcinoma of the peritoneum (PSCP) may be multifocal in origin. Utilizing a PCR based method to detect tandem repeat polymorphisms in formalin fixed tissue, loss of heterozygosity at eight loci on chromosomes 1, 3, 4, and 17 was studied in six cases of PSCP. Loss of heterozygosity was assessed at between 5 and 11 tumor sites/patient. Allelic losses at 4 loci (1q32–qter, 3p14.3-21.1, 17q12, 17q21.3-23) were noted. Three cases demonstrated a different pattern of allelic loss at various anatomic sites within the same patient. In an additional case, a mutation of the p53 gene, detected by quantitative PCR followed by single-strand conformation polymorphism analysis, was detected in only 2 of 5 tumor sites. The pattern of allelic loss and the mutational pattern of the p53 gene varied at tumor sites within the same patient in 4 of 6 cases of PSCP. These findings are consistent with histopathological evidence that PSCP is multifocal in origin.

Introduction

PSCP is a malignancy occurring exclusively in women which is histologically indistinguishable from papillary serous carcinoma of the ovary (1). The disease diffusely involves peritoneal surfaces, while sparing or superficially involving the ovaries (2). It may develop years after oophorectomy for benign disease or after prophylactic oophorectomy for a family history of ovarian cancer (3, 4). The pathogenesis of PSCP remains controversial. Some think that the malignancy arises from ovarian surface epithelium with subsequent i.p. spread, while others theorize that PSCP may have a multifocal origin in rests of müllерian epithelium (5). Clinicopathological studies support a multifocal origin of PSCP, but such studies are not conclusive (6). The purpose of this study is to determine whether the origin of PSCP is multifocal by comparing genetic changes at multiple i.p. sites. Two molecular genetic methods were used: an examination of the pattern of allelic loss on chromosomes 1, 3, 4, and 17; and a study of the mutational pattern of the p53 gene. According to the monclonal theory of carcinogenesis, cells within a tumor are derived from a single transformed cell. Genetic changes within that single cell would be, therefore, transmitted to all progeny. We have previously demonstrated in invasive epithelial tumors of the ovary that the pattern of allelic loss, p53 mutation, and X chromosome inactivation are identical at primary and metastatic sites (7). These observations support a unifocal origin for ovarian epithelial malignancy. The purpose of the current investigation is to extend these observations to PSCP.

Materials and Methods

DNA Extraction. DNA was extracted from archival material which had been previously fixed in formalin and embedded in paraffin. A total of six cases of PSCP were used in this study. The histopathological diagnosis was confirmed by a review of hematoxylin-eosin stained sections. All cases were characterized by disseminated papillary serous carcinoma in the presence of normal sized ovaries (mean size, 2.2 × 1.6) which either were entirely uninvolved or had small capsular implants with little or no stromal involvement. In blocks containing tissue with focal tumor involvement, the tumor area was circled and trimmed from the block prior to DNA extraction. DNA extracted from uninvolved cervical stroma, round ligament, or bowel serosa was used as a "normal control." DNA extraction was performed as described previously (8, 9).

Tandem Repeat Polymorphism Analysis. Loss of heterozygosity was studied by PCR amplification of specific alleles containing dinucleotide tandem repeat polymorphisms. The D3S1007, D4S127, THRB, and MPO primers were purchased from Research Genetics (Huntsville, AL) and the DIS103, SIS104, and DIS106 primers were from the American Type Culture Collection (Rockville, MD). Markers were selected based upon the frequency of allelic loss reported in epithelial ovarian cancer. A high frequency of allelic loss has been reported in epithelial cancer on chromosomes 3 and 17, whereas a low frequency of allelic loss has been reported on chromosomes 1 and 4. Only one of two primers for each locus was end-labeled with 32P ATP using T4 polynucleotide kinase (Boehringer Mannheim, Indianapolis, IN). The PCR conditions were: for D17S250, DIS103, and DIS104, 27 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 2 min; for THRB, 30 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 2 min; for D3S1007, 35 cycles of 94°C for 1 min, 60°C for 2 min, 72°C for 1 min; for DIS103, 35 cycles of 94°C for 1 min, 60°C for 2 min, 72°C for 1 min; for DIS106, 35 cycles of 94°C for 1 min, 55°C for 2 min, 72°C for 1 min, and finally for MPO, 27 cycles of 94°C for 1 min, 45°C for 2 min, and 72°C for 2 min. Forty-five μl of formamide dye were added to the PCR product at the end of the reaction, and 3 μl were loaded onto a preheated 8% (for THRB and D17S250) or 6% (D3S1007, D4S127, MPO, DIS106, DIS103, DIS104) denaturing polyacrylamide gel (acrylamide:bisacrylamide, 19:1) and electrophoresed at 1700 V. The gel was dried and exposed to X-ray film at −70°C for 6–12 h.

Direct Cycle Sequencing of PCR Product. The bands containing a p53 mutation as indicated by a mobility shift on the SSCP gel were cut from the gel and reamplified as described previously (8) (using the same primers and reaction cycle conditions). PCR products were purified on a 5% nondenaturing polyacrylamide gel and sequenced according to the method of Mok et al. (12). The sequences were confirmed by sequencing both sense and antisense cDNA strands in an 8% polyacrylamide gel containing 7 μm urea.

Results

Eight cases of PSCP were diagnosed and treated at Brigham and Women's Hospital between 1990 and 1992. Appropriate quantities of tissue were available in six of the cases. The mean age of onset was 63.3 years (range, 57–74 years). All cases were of advanced Interna-
MULTIFOCAL CARCINOMA OF THE PERITONEUM

Table 1 Pattern of allelic loss at multiple tumor sites in patients with PSCP

<table>
<thead>
<tr>
<th>Primer</th>
<th>Map location</th>
<th>E1</th>
<th>E2</th>
<th>E4</th>
<th>E5</th>
<th>E6</th>
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<td>00000000</td>
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<td>1q22-qter</td>
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*0, heterozygous; n, noninformative; 1, 2, loss of allele 1 or 2.

In patient E5 there was a selective loss of allele 2 at 1 of 11 tumor sites in the region of 3p14.3-21.1. Finally, in patient E8 there was a selective loss of allele 1 at 2 of 5 tumor sites in the region 3p14.3-21.1 and in 3 of 5 tumor sites at 17q21.3-23.

Losses in patients E1, E4, E5, and E8 are displayed in Fig. 1. Therefore, there was evidence of selective allelic loss in four of the six cases studied.

All six cases were screened for p53 mutations. Five of the cases had no detectable mutations. Only one of the six cases (E8) demonstrated a p53 mutation by SSCP analysis. This mutation was located in exon 7 of the p53 gene as detected by PCR-SSCP analysis (case E8). T1, left ovary; T2, left tube; T3, omentum; T4, uterine serosa; N, normal cervix.

Fig. 2. Mobility shift of mutated exon 7 of the p53 gene as detected by PCR-SSCP analysis (case E8). T1, left ovary; T2, left tube; T3, omentum; T4, uterine serosa; N, normal cervix.

Fig. 3. Sequencing of the mutated exon 7 of the p53 gene. T1, left ovary; T3, omentum; N, normal cervix. Arrowhead, site of point mutation.
This report describes the first study utilizing molecular biological techniques aimed at determining whether or not PSCP is a multifocal disease. We have demonstrated previously that in advanced stage epithelial ovarian cancer, the pattern of allelic loss, X chromosome inactivation, and p53 mutation are all consistent with a unifocal origin for these more common tumors (7). We have demonstrated that among six cases of documented PSCP, using a strict definition of the disease and with meticulous pathology review and microdissection from paraffin block, four cases show evidence of being multifocal in origin. Four cases have a different pattern of allelic loss at various anatomic sites, and one of these cases also has a p53 mutation present in some but not all anatomic sites.

A possible explanation for the distinctly different findings in these two forms of malignancy is that PSCP arises as synchronous primaries within multifocal rests of müllerian epithelium, scattered throughout the peritoneal cavity, whereas the more common forms of advanced ovarian cancer do not (1). There is a considerable body of histopathological evidence supporting the potential of the female upper genital tract to develop independent primary neoplasia at multiple anatomic sites (2). For example, Russell et al. (5) have demonstrated that many serous and endometrioid tumors of the ovary are associated with biopsy proved second primaries, as in the case of stage IB ovarian cancer or endometrioid ovarian cancer occurring with endometrioid adenocarcinoma of the endometrium.

An alternative theory for the pathogenesis of PSCP is that it is not a distinct entity but a variant of serous carcinoma of the ovary in which the primary ovarian lesion is not clinically apparent. It is difficult to explain, however, why patients can develop PSCP up to 30 years following oophorectomy for benign disease or after prophylactic oophorectomy. Extending the observations to PSCP, we interpret our observations as evidence of multifocal development.

The occurrence of PSCP following prophylactic oophorectomy in women with a strong family history of ovarian cancer has been reported. Some of these cases have been discounted by the identification of microscopic foci of invasive carcinoma in an inadequately sampled ovaries, but most reports are credible. Our report includes two women with a documented family history of ovarian cancer in first degree relatives, one of whom (El) has molecular genetic evidence of multifocal origin of her PSCP tumor. These findings raise troubling questions about the role of screening sonography and serum tumor markers for early detection of ovarian neoplasms among these high risk patients with a strong family history.

References
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