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Frequent Microsatellite Instability in Epithelial Borderline Ovarian Tumors

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Abstract

To further define the genetic events that could lead to the development of borderline ovarian tumors (BOTs), we analyzed 13 microsatellite markers on chromosomes 3p and q in 18 BOTs and compared the results to 31 serous invasive epithelial ovarian cancers (IEOCs). Five of the 18 BOTs showed microsatellite instability (MSI) at one or more loci, compared to only 2 of the 31 IEOCs studied (P < 0.04). In two of these five BOTs, MSI was found in multiple loci. All BOTs with MSI were serous, while none of the mucinous type showed any alteration. Loss of heterozygosity was found in only 1 of the 18 BOTs, but in 12 of the 31 IEOCs (P < 0.01). This first report of a relatively high percentage of MSI in BOTs opens a wide spectrum of new hypotheses for borderline ovarian tumorigenesis as well as several new research avenues.

Introduction

BOTs are well-recognized entities with intermediate characteristics between benign and malignant ovarian neoplasms in regard to atypicality and clinical behavior. They display a greater degree of epithelial proliferation and atypia than benign epithelial ovarian tumors. However, they lack stromal invasion, compared with IEOCs, and the prognosis of patients with BOT is remarkably better (1).

It is now widely accepted that human neoplasms are the end result of a multistep process, in which accumulation of several genetic alterations plays a definite role. At least three types of cancer-related genes have been recognized: tumor suppressor genes, oncogenes, and mismatch repair genes. An accumulation of mutations within these genes is associated with a variety of human cancers. In epithelial ovarian neoplasms, and especially BOTs, these genetic changes are poorly understood. IEOCs display LOH at multiple chromosomal sites, suggesting that inactivation of multiple tumor suppressor genes plays a role in the etiology of this malignancy. Overexpression of oncogenes such as Her-2/neu and K-ras has also been associated with IEOCs (2). On the other hand, BOTs have failed to show LOH at several chromosomal sites, suggesting that this mechanism may not be involved in the development of this disease (3–6). However, it has been shown that these tumors display a high rate of K-ras mutation (7).

MSI is widely accepted as an indicator of loss of DNA mismatch repair activity in human tumor cells (8). However, in IEOCs, MSI has been rarely observed (9). Furthermore, BOT has not been shown to have MSI in the limited number of loci that have been screened. In this study, we analyzed 11 loci on chromosome 3p and 2 loci on chromosome 3q in 31 IEOCs and 18 BOTs, as part of a systematic survey of all chromosomes, to further define the spectrum of genetic abnormalities associated with epithelial ovarian tumors.

Materials and Methods

Specimen Collection and DNA Extraction. Surgical specimens of 31 IEOCs and 18 BOTs were obtained from previously untreated patients following a protocol approved by the Human Subject Committee of Brigham and Women’s Hospital. Segments of normal fallopian tube, uninvolved round ligament, or peripheral blood were obtained from the same patients to serve as normal tissue controls. All histopathological diagnoses of invasive and borderline epithelial ovarian tumors were confirmed and graded by a gynecological pathologist (W. R. W.), and all cases were surgically staged according to the International Federation of Gynecology and Obstetrics criteria (10). All 31 invasive cases examined were papillary serous adenocarcinomas of advanced stage and grade (stage III or IV, grade 2 or 3). The 18 borderline tumors included 14 serous and 4 mucinous, stages I–III. DNA was extracted using previously published methods (11).

PCR Amplification and Analysis. The PCR was performed to amplify regions of dinucleotide repeats on chromosome 3 using 13 oligonucleotide primer pairs (Research Genetics, Huntsville, AL). The primers and their corresponding map positions are summarized in Table 1. PCR analysis was performed according to previously published methods (12). Briefly, the forward primer was end radiolabeled with T4 polynucleotide kinase (Boehringer Mannheim, Indianapolis, IN) and )-32P-ATP. The reaction mix was then diluted into a final volume of 318 plimer-PCR mixture containing 40 µl 10× PCR buffer (0.1 M Tris-HCl and 0.5 M KCl, pH 8.3), 18–50 mM MgCl2, 18 µl 2.5 mM dNTPs, and 2 µl (10 units) of Taq polymerase (Perkin Elmer/Cetus, Norwalk, CT).

Amplification was carried out with 50 ng genomic DNA using 35 cycles of PCR (at 95°C for 1 min of denaturation, 50–65°C for 2 min of annealing, and 72°C for 1 min of elongation). Forty-five µl of loading buffer containing 95% formamide, 18 µl EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol FF (Sigma, St. Louis, MO) were added to the PCR product. Three µl of the mixture were loaded onto a 6% polyacrylamide gel and run at 1700 V. The gel was then dried and exposed to X-ray film with an intensifying screen. MSI was defined by a mobility shift in the tumor DNA band as compared to the normal DNA band detected. LOH was defined as a visible reduction of 50% or more in the band intensity of the tumor sample when it is compared to the corresponding normal tissue band.

Statistical analysis to compare the rate of LOH and MSI on BOTs and invasive ovarian cancer was performed using Fisher’s exact test.

Results

DNA samples from 31 patients with IEOCs and 18 patients with BOTs were analyzed with PCR using 11 microsatellite markers from chromosome 3p and 2 markers from 3q. The percentage of LOH and MSI obtained from each locus in borderline and invasive ovarian tumors is summarized in Table 2.

Five (27%) of the 18 BOTs showed MSI in at least one locus (Fig. 1A). Among these cases, tumors 354 and 474 demonstrated instability at multiple loci. Examples of MSI observed in borderline tumors are shown in Fig. 2A. The MSI was present at only five of the loci tested, specifically PMS2, D3S1291, D3S1323, D3S1428, and D3S1452 in one or more of the 18 BOTs.
but they were widely spread over both arms of chromosome 3. Four of the five tumors showed MSI at D3S966 on 3p21.3, and three of five serous cell type. None of the four mucinous borderline tumors tested showed LOH in either all or a majority of the informative PCRs and gel loadings. Only one tumor showed LOH at one single marker (tumor 403).

Discussion

In this study we have detected MSI on chromosome 3 in 27% of BOTs but in a significantly lower percentage of IEOCs. Abnormalities in microsatellites have been implicated in the genesis of several neurological diseases and human neoplasms (13, 14). Current data suggest that mutations in at least one of four human mismatch repair genes, hMSH2, hMLH1, hPMS1, and hPMS2, are involved in the genesis of those tumors exhibiting MSI (8). Extensive studies on MSI and mismatch repair gene mutations have been performed on hereditary nonpolyposis colorectal cancer and extracolonic tumors (including IEOC) found in hereditary nonpolyposis colorectal cancer kindreds (Lynch II syndrome). More than 80% of these tumors demonstrated a high frequency of MSI (15). However, with the exception of IEOC arising in the Lynch II syndrome, mutations in mismatch repair genes have rarely been identified in sporadic IEOCs (16). Furthermore, BOTs have not been identified in the Lynch II syndrome, and MSI has not been previously reported in BOTs. In this study, we have found MSI on chromosome 3 in 5 of 18 borderline tumors. This finding suggests that mutations in mismatch repair genes may be responsible for the development of a proportion of BOTs. Although this remains to be confirmed, similarities between the behavior of BOTs and those tumors associated with mutations in mismatch repair genes are striking. Several reports have shown that tumors demonstrating MSI tend to behave indolently and have better survival rates than similar tumors that do not display this abnormality. Additionally, they tend to be of a lower grade (17, 18). Furthermore, it has been shown that tumors demonstrating MSI do not show LOH, suggesting that allelic loss is probably not involved in their development. In addition, these tumors are generally of earlier onset (8). BOTs demonstrate all of these characteristics. They arise earlier, are less aggressive, of lower grade, and have better prognosis than invasive ovarian cancer. Furthermore, it has been previously shown that LOH is not associated with BOTs (2). The finding that MSI is associated with BOT but not with invasive ovarian cancer strongly suggest that they have different molecular etiologies.

If defective mismatch repair genes are responsible for the development of BOT, more MSI in other chromosomal sites should have been reported in previous studies, according to the "mutator phenotype" model proposed by Loeh (19). Microsatellite analysis has been performed in BOTs in a limited number of chromosomal sites. However,
**Fig. 1.** Summary of results obtained at each of the loci analyzed. A, BOTs; B, invasive ovarian cancer. LOH; heterozygous, no alterations; MSI; homozygous, no alterations; N, no results.

MSI has not been previously identified. Dodson *et al.* (20) analyzed one to eight microsatellite markers on each human chromosomal arm in four BOTs showing no evidence of MSI. Eccles *et al.* (21) studied two markers on chromosome 17p and one on 17q in a larger number of BOTs without reporting MSI either. Several studies of BOTs, including some of the borderline tumor samples used in the present
study, were performed in our laboratory on different chromosomal arms. Rodabaugh et al. (3, 6) studied 12 loci on chromosome 6q and 12 loci on 9p. Wertheim et al. (6) and Tangir et al. (5) studied 4 loci on chromosome 17p and 18 loci on 17q. None of these studies showed evidence of MSI in borderline tumors (3—6). When taken together with our findings, this report suggests that MSI in BOT is probably a focal more than widespread phenomenon. It has been described previously in colorectal tumors that approximately 14% of these tumors show alterations at a minority of loci, possibly reflecting a different phenotype than those showing MSI at the majority of loci tested (22). Furthermore, Ogasawara et al. (23) reported a relatively high incidence of MSI on chromosome 3p in esophageal squamous cell carcinoma but a low incidence on other chromosomes. Extensive screening for MSI using markers on different chromosomal arms in BOT is currently underway in our laboratory.

Interestingly, the five tumors that showed MSI in this report were serous BOTs, and none of the four mucinous tumors examined showed microsatellite alterations. Although the difference was not statistically significant, possibly due to the small number of mucinous tumors, the suggestion that MSI is involved in the tumorigenic pathway of serous but not in mucinous BOT is intriguing. This finding further supports the hypothesis that these two histopathological types of BOTs might be different biological entities (7, 24).

In contrast to BOT, invasive ovarian cancer demonstrated a significantly lower rate of MSI on chromosome 3. Reports of MSI in invasive ovarian cancer have been contradictory. Boyer et al. (25) and Orth et al. (26) have found a high frequency of MSI in ovarian cancer cell lines. However, in ovarian cancer tissue, MSI is rarely observed (9). In a recent study, King et al. (27) have found a high percentage of MSI in uncommon types of epithelial ovarian cancer such as endometrioid or mixed serous and mucinous types, and proposed that the incidence of MSI in ovarian cancer may vary between different histopathological types. Fujita et al. (16) were able to demonstrate mutations at the hMSH2 gene in a proportion of endometrioid ovarian cancer cases that showed MSI. The involvement of MSI and mismatch repair genes in the development of uncommon epithelial ovarian cancer warrant further study.

In this study we also reported that 39% of invasive ovarian cancers showed LOH at one or more loci on chromosome 3. This is in accordance with previous studies showing high percentages of LOH on chromosome 3 in invasive ovarian cancer (28—30). Based on the pattern of LOH, a minimal region of loss unit was defined at locus D3S1007 on 3p25, where a tumor suppressor gene involved in the genesis of invasive ovarian cancer could be located. This region has not been described previously as lost in ovarian cancer. However, it has been found as commonly lost in small cell lung cancer (14) and in breast cancer (31). The LOH pattern in invasive ovarian cancer also defined a second region of common loss at D3S966 on 3p21.3. Jones and Nakamura (30) analyzed four ovarian tumors describing a common region of loss at 3p21.1—22, which could be the same region described here. Sato et al. (29) analyzed chromosome 3p using four RFLP markers spanned between 3p12 and 3p22.3 and found a maximum LOH rate of 18%, but could not define any minimal region of loss. The discrepancy between these two earlier reports and our findings may probably be due to our use of more markers with more precise chromosomal localization. The detailed deletion map allowed us to identify a new region of common loss on 3p25 in ovarian cancer and to further define the region previously described on chromosome 3p21.3. This result suggests the presence of one or more tumor suppressor genes on chromosome 3p, which is further supported by Rimessi et al. (32), who demonstrated that the introduction of normal copies of human chromosome 3 into the HEY ovarian carcinoma cell line could induce growth arrest. The region on 3p21.3 has also been previously described as commonly lost in squamous cell carcinoma of the head and neck, uterine cervix, and lung, and LOH at 3p25 has been associated with lymph node metastasis in esophageal squamous cell carcinoma (23). These data suggest that inactivation of common tumor suppressor genes could play a role in the development of different human cancers.

We found a high percentage of MSI on chromosome 3 in serous BOT but not in mucinous BOT and invasive ovarian cancer, suggesting that: (a) BOT and invasive ovarian cancer are different entities; (b) serous and mucinous BOT arise through different biological pathways; and (c) inactivation of mismatch repair genes may be involved in the development of serous BOT. In addition, we identified a novel region of common loss on chromosome 3p25 in invasive ovarian cancer, suggesting the presence of another tumor suppressor gene on chromosome 3p involved in the development of this disease.

**References**


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