Localisation of an Ovarian Cancer Tumor Suppressor Gene to a 0.5-cM Region between D22S284 and CYP2D, on Chromosome 22q

Emma J. Bryan, Richard H. Watson, Michael Davis, Andrew Hitchcock, William D. Foulkes, and Ian G. Campbell

Obstetrics and Gynaecology, University of Southampton, Princess Anne Hospital, Caxford Road, Southampton SO16 5AY [E. J. B., R. H. W., M. D., I. G. C.]; and Department of Histopathology, Southampton General Hospital, Southampton [A. H.], United Kingdom; and Division of Medical Genetics, Department of Medicine, Montreal General Hospital, Montreal, Quebec, Canada H3G 1A4 [W. D. F.]

Abstract

The detection of loss of heterozygosity, indicative of the presence of a tumor suppressor gene, has been reported to occur frequently on chromosome 22q in human ovarian cancer. In this study, 110 sporadic ovarian tumors were analyzed using 8 polymorphic loci to define a minimum region of loss. Fifty-eight (53%) tumors showed loss of heterozygosity, and of these 6 exhibited partial loss, enabling the identification of two candidate tumor suppressor gene loci. One region, of less than 0.5 cM, is flanked by D22S284 and CYP2D, and a second region lies distal to D22S276. Analysis of loss of heterozygosity with respect to grade and stage suggests that chromosome 22q loss of heterozygosity is of more relevance in tumor progression rather than initiation.

Introduction

In ovarian cancer, LOH\(^1\) on chromosome 22q has been reported by a number of groups, with frequencies ranging between 25 and 71% (1–5). In a previous report, we confirmed that LOH on 22q is a frequent event in ovarian cancers and indicated that the putative TSG was located distal to D22S283, which corresponds to 22q12–qter (6). This region is also thought to contain TSGs of relevance in a number of other malignancies including meningioma (7), hepatocellular carcinomas (8), breast cancers (9–11), and colon carcinomas (12–14). This study was designed to further clarify the relevance of LOH on this chromosome in ovarian carcinogenesis and refine the minimum region(s) of LOH. To achieve this we have analyzed 110 ovarian tumors for LOH on 22q using 8 microsatellite markers spanning 22q12–ter.

Materials and Methods

Tumor Specimens and DNA Extraction. One hundred fresh tumor and matching blood samples were obtained from patients undergoing surgery for primary ovarian cancer in hospitals in and around London, and in the Wessex region. Additionally, 10 low-grade and/or early-stage tumors were accessed from paraffin blocks. The collection included 48 serous tumors, 21 mucinous, 20 endometrioid, 9 undifferentiated, 2 granulosa cell tumors, 1 adenocarcinoma, 4 mixed Müllerian tumors, and 5 unspecified. Fresh tumors were snap frozen and stored in liquid nitrogen until the DNA was extracted using the salt chloroform method described by Müllenhoff et al. (15). DNA from paraffin blocks was extracted from 5-mm sections cut from formalin-fixed, paraffin-embedded sections. These were heated onto slides at 60°C for 10 min and then dewaxed using 2 washes each of xylene and ethanol. After air drying, one side was stained by hematoxylin and eosin to allow the tumor areas to be visualized. This slide was used as a template to scrape the tumor areas from a nonstained slide using a 25-gauge needle. Matching normal cells were obtained by the same method from normal areas from the same section. DNA was then extracted using commercially available DNA extraction columns (Qiagen).

PCR and Microsatellite Analysis. PCR was carried out using 10–200 ng of genomic DNA in a reaction volume of 20 μl, with the inclusion of 1 μCi of [γ-\(^32\)P]dCTP (16). Eight microsatellite markers were used to study LOH: D22S283; PDGFB; D22S299; D22S284; CYP2D; D22S276; D22S282; and D22S274 (17). The alleles were separated on nondenaturing 5–8% polyacrylamide gels and examined after autoradiographic exposure. Assessment of LOH was based on visual comparison of the intensities of the normal blood DNA alleles and those of the tumor.

Results

LOH on Chromosome 22q. LOH analysis was performed on 110 tumors using up to 8 polymorphic microsatellite markers. LOH at one or more loci was detected in 58 of 110 (53%) tumors, with 52 showing LOH with all informative markers. Six tumors demonstrated LOH on only part of chromosome 22q, as shown in Table 1. Some of the key autoradiograph results for tumors 63, icrf28, and icrf72 are shown in Fig. 1. Tumors 63 and 78 show retention of heterozygosity for CYP2D but LOH for all proximal loci. Tumors 139 and 187, both show retention of the most distal locus (D22S274) and LOH for all informative markers. Tumor icrf72 has two regions of LOH, the most interesting of which is an interstitial deletion centered on the CYP2D locus. The pattern of losses in these tumors is consistent with two distinct regions of LOH as shown in Fig. 2. One is located between D22S284 and CYP2D2, which is a genetic distance of approximately 0.5 cM. The second is flanked proximally by D22S276, but the distal boundary is uncertain because it is not known which region or regions the deletions in tumors 139 and 187 are targeting. Microsatellite instability was detected in tumors 63 and 139 using marker D22S276 but was not observed with any of the other microsatellite markers.

LOH with Respect to Grade, Stage, and Histological Type. Analysis of LOH with respect to tumor grade (Table 2) and tumor stage (Table 3) reveals an increasing frequency of LOH in both higher-grade and later-stage tumors, with relatively few low-grade or early-stage tumors showing LOH. In particular, only 19% of grade 1 tumors showed LOH compared with a striking 84% of grade 3 tumors, suggesting that 22q LOH is a late event in ovarian tumorigenesis. The frequency of LOH according to histological subtype is shown in Table 4. LOH was common in serous and endometrioid tumors but detected in only 2 of 21 mucinous tumors. It was of interest that all 4 mixed Müllerian tumors exhibited LOH.

Discussion

Consistent with our earlier study (6), we have shown LOH on 22q to be a very frequent event in ovarian carcinomas. The large size of our tumor collection (none of the previous studies looked at more than 50 tumors), and the high frequency of allele loss detected (up to 84%
in grade 3 tumors), lend weight to the existence of an important TSG(s) on chromosome 22q.

In our previous study, we determined the candidate TSG region to be distal to D22S283. The data presented here are consistent with that location and have enabled a refinement of one TSG loci to a 0.5-cM region flanked by D22S284 and CYP2D. A second TSG loci may also be present in the region distal to D22S276, a genetic distance of >6 cM. The distal boundary of this region may lie above D22S274 because tumors 1, 39 and 1, 87 show retention of this locus, but the possibility that the deletions in these tumors are targeting the proximal TSG region cannot be excluded.

The candidate regions identified in this study overlap with those identified in other cancers as illustrated in Fig. 2. It is interesting to note the overlap with the candidate region identified in colorectal carcinomas. Because these malignancies are both epithelial derived, there is a possibility that these deletions are targeting the same TSG. Candidate loci for breast (11) and hepatocellular tumors (8) have not been defined sufficiently to make any meaningful comparisons.

Our findings suggest that loss of chromosome 22q is an intermediate event in ovarian tumorigenesis. LOH was very frequent in grade 3 tumors compared with either grade 1 or grade 2 tumors. With respect to histological subtype, LOH on 22q appears to be of particular relevance in serous tumors. It was of interest that all 4 of the mixed Müllerian tumors studied showed LOH; this warrants further investigation in a larger sample of tumors. In contrast, LOH was especially uncommon in mucinous tumors. Increasingly, it appears

<table>
<thead>
<tr>
<th>Tumor number</th>
<th>63</th>
<th>78</th>
<th>139</th>
<th>187</th>
<th>icrf72a</th>
<th>icrf72b</th>
</tr>
</thead>
<tbody>
<tr>
<td>S283</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>HET</td>
<td>HET</td>
</tr>
<tr>
<td>PDGFB</td>
<td>LOH</td>
<td>LOH</td>
<td>LOH</td>
<td>LOH</td>
<td>HET</td>
<td>HET</td>
</tr>
<tr>
<td>S299</td>
<td>LOH</td>
<td>LOH</td>
<td>LOH</td>
<td>LOH</td>
<td>HET</td>
<td>HET</td>
</tr>
<tr>
<td>S284</td>
<td>LOH</td>
<td>LOH</td>
<td>LOH</td>
<td>LOH</td>
<td>HET</td>
<td>HET</td>
</tr>
<tr>
<td>CYP2D</td>
<td>HET</td>
<td>HET</td>
<td>HET</td>
<td>HET</td>
<td>LOH</td>
<td>LOH</td>
</tr>
<tr>
<td>S276</td>
<td>MI</td>
<td>MI</td>
<td>MI</td>
<td>MI</td>
<td>LOH</td>
<td>LOH</td>
</tr>
<tr>
<td>S282</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>LOH</td>
<td>LOH</td>
</tr>
<tr>
<td>S274</td>
<td>NI</td>
<td>NI</td>
<td>HET</td>
<td>HET</td>
<td>LOH</td>
<td>LOH</td>
</tr>
</tbody>
</table>

*a Tumors prefixed with icrf were collected from hospitals in and around London, and the remaining tumors were collected from hospitals in the Wessex region.

*b NI, not informative due to constitutional homozygosity; HET, constitutional heterozygosity without loss; LOH, loss of constitutional heterozygosity; MI, microsatellite instability; ND, not done.

c For simplicity, the D22 designation has been omitted from the marker names.
that mucinous tumors differ fundamentally from the other histological sub-types and in general show fewer genetic alterations (18), apart from an increased prevalence of RASK mutations (19).

In conclusion, this study has provided further evidence for the presence of important ovarian TSGs on chromosome 22q. The most proximal candidate region between D22S284 and CYP2D is now a Numerator is the number of tumors of the specified histological type with LOH at any locus on chromosome 22q. Denominator is the number of informative tumors of that histological type.

Acknowledgments

The authors thank Sister Thomas and Sister Barron, who collected the tumors from hospitals in Wessex.

References


Localization of an Ovarian Cancer Tumor Suppressor Gene to a 0.5-cM Region between D22S284 and CYP2D, on Chromosome 22q
