Genomic Alterations in Fallopian Tube Carcinoma: Comparison to Serous Uterine and Ovarian Carcinomas Reveals Similarity Suggesting Likeness in Molecular Pathogenesis

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Abstract

Serous carcinomas of the fallopian tube, uterus, and ovary resemble each other both histologically and in clinical behavior. Comparative genomic hybridization was performed on 20 primary fallopian tube carcinoma specimens to find regions of the genome involved in tubal carcinogenesis and to compare the genomic alterations with those previously detected in serous ovarian and uterine carcinomas. The most frequent changes detected in fallopian tube carcinoma were gains at 3q (70%) and 8q (75%), with high-level amplifications in several cases. Other common gains occurred at 1q, 5p, 7q, 12p, and 20q. The most frequent losses were found at 18q, 8p, 4q, and 5q. The frequency and the pattern of chromosomal changes detected in tubal carcinoma were strikingly similar to those observed in serous ovarian and uterine carcinomas, suggesting common molecular pathogenesis.

Introduction

Gynecological carcinomas are classified primarily according to their organ of origin. However, carcinomas of the ovary, uterus, and fallopian tube exhibit various histologies including serous, endometrioid, and mucinous subtypes, whose risk factors and biological and clinical behavior are dissimilar (1-3), suggesting that they might be distinct entities.

Molecular genetic analyses have provided some background to the observed phenotypic differences. Frequent P53 mutations have been detected in serous uterine and ovarian carcinomas whereas they are rare in other histological subtypes (4, 5), and K-RAS mutation is frequent in mucinous ovarian carcinomas, but infrequent in the serous subtype (6). Different histological subtypes of uterine (endometrioid or serous) and ovarian (serous, endometrioid, or mucinous) carcinomas differ in respect to their genomic alterations detected by CGH (7, 8). Furthermore, our previous results suggested that the same histological subtypes of tumors in the uterus and the ovary have similar patterns of genomic abnormalities (7, 9), but the possible kindred of the same histological subtype of tumors from the ovary, uterus, and fallopian tube has not been studied systematically.

Serous carcinoma constitutes approximately 95%, 55%, and 10% of tubal, ovarian, and uterine carcinomas, respectively. Compared with uterine and ovarian carcinomas, primary fallopian tube carcinoma is rare. Only few investigations have been carried out on the genetic background of fallopian tube carcinoma (10, 11), and its pathogenesis is poorly understood.

We performed CGH analysis on 20 primary fallopian tube carcinomas. The results were compared with the changes in serous uterine and ovarian carcinomas reported earlier (7, 9). Our aims were: (a) to identify the chromosomal regions involved in the pathogenesis of fallopian tube carcinoma; and (b) to find out whether the patterns of genetic aberrations detected in serous fallopian tube, uterine, and ovarian carcinomas are similar, which would suggest a common molecular pathogenesis for this group of gynecological carcinomas.

Materials and Methods

Tumor Specimens. The material consisted of 20 primary fallopian tube carcinomas treated at the Department of Obstetrics and Gynecology, Helsinki University Central Hospital. All of the tumors were histologically serous (Table 1). Histology of the specimens was confirmed by the same investigator (R. B.), and only tissue samples containing more than 50% of tumor cells by histological examination were included in the study. DNA extraction was performed using standard protocols from either paraffin-embedded blocks or frozen sections. The CGH findings of tubal carcinomas were compared with the changes seen in 24 serous endometrial (7) and 20 serous ovarian carcinomas (9), analyzed earlier. The stages and grades of the reference material were similar to those in the tubal carcinoma group.

CGH. The protocol for directly fluorochrome-conjugated nucleotides was followed with some modifications (9). Briefly, 1 µg of tumor DNA was labeled with FITC-12dUTP and FITC-12dCTP (1:1; DuPont), and 1 µg of normal DNA was labeled with Texas Red-5dUTP and Texas Red-5dCTP (1:1; DuPont) in standard nick translation. Equal amounts of labeled test and reference DNA were hybridized to normal metaphase spreads. The slides were counterstained with 4,6-diamidino-2-phenylindole (DAPI; Sigma Chemical Co., St. Louis, MO) for the identification of the chromosomes.

The results were analyzed using an Olympus fluorescence microscope and an ISIS digital image analysis system (MetaSystems GmbH, Altusheim, Germany). Three-color images (green for tumor DNA, red for normal reference DNA, and blue for DNA counterstain) were acquired from 6-10 metaphases/sample. Green:red ratio profiles along the chromosome axis were displayed. The chromosomal regions with green:red ratio exceeding 1.17 were considered to be overrepresented (gains), whereas the regions with a ratio below 0.85 were considered underrepresented (losses). These values were set on the basis of the results of negative control experiments in which two differently labeled normal DNAs were hybridized together. In the negative controls, the ratios varied within these limits. Tumor DNA with the known copy number changes was used in positive control experiments. All findings were confirmed using a confidence interval of 99%. The cutoff level for high-level amplification was 1.5. Telomeric, and heterochromatic areas were discarded from the analysis.

Results

DNA sequence copy number changes were detected in all 20 fallopian tube carcinoma specimens (mean, 7.0 aberrations/tumor; range, 1-25; Table 1). Gains were more frequent than losses (1.6:1). The most common regions of increased copy number were 3q

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4 The abbreviations used are: CGH, comparative genomic hybridization; LOH, loss of heterozygosity.
Table 1—Histopathological and clinical characteristics and CGH findings in 20 primary fallopian tube carcinomas

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Stage</th>
<th>Grade</th>
<th>Copy number changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>137</td>
<td>la</td>
<td>3</td>
<td>+4q25-q31.1, +7q31-qter, +8q21.1-qter, +12q12-qter</td>
</tr>
<tr>
<td>223</td>
<td>Ia</td>
<td>3</td>
<td>+1p33-p1ter, +3q25-qter, -5q14-q21, +10p, +12p, -18q12-qter, +19, +20</td>
</tr>
<tr>
<td>268</td>
<td>Ille</td>
<td>2</td>
<td>-4q22-qter, +5p, -5q, -Xp11.2-qter, +8q22-qter, +9p, -9q, -10, +12qcen-p12, -13q, -14q21-qter, -15qcen-q22, -16q, -17, -18, +20q11.2-qter</td>
</tr>
<tr>
<td>601</td>
<td>Ille</td>
<td>3</td>
<td>+1p22-p34.3, -2q32-q36, +3q24-qter, -4q12-qter, +5p15.1-p1ter, -5q13-q23, -X, +6p, -6q22-qter, +7q32-qter, -8p, +8q, -9p13-qter, -11p14-qter, +11p11.2-qter, +12p, -13q21-qter, -18q21-qter, +19, +20</td>
</tr>
<tr>
<td>603</td>
<td>Ille</td>
<td>1</td>
<td>-3p12-p14, +3q13.1-qter, -6p, -7p14-p1ter, +7q22-q31, -7q32-qter, -8p12-pter, +8q8q12-qter, -15, -17p, -18q21-qter</td>
</tr>
<tr>
<td>605</td>
<td>Ille</td>
<td>2</td>
<td>+1, +2, +3q, +5p13-qter, -X, +6, +7, +8p, +8q11-p1ter, +10p10-q21, +13q22-qter, -17p10-q12, +22</td>
</tr>
<tr>
<td>606</td>
<td>Ille</td>
<td>2</td>
<td>+1q24-qter, +3q25-qter, -4, -5q12-qter, -6, -9</td>
</tr>
<tr>
<td>607</td>
<td>Ila</td>
<td>2</td>
<td>+1q32-q42, +13q22-q33</td>
</tr>
<tr>
<td>608</td>
<td>Ille</td>
<td>3</td>
<td>+3q21-q26.1, -8p, +8q23-qter, -18, -22</td>
</tr>
<tr>
<td>610</td>
<td>Ille</td>
<td>3</td>
<td>+1q21-q24, +3q13.1-qter/3q21-qter, +5p, +8q13-q24.1, +10p11.2-qter, -15q, +20q92</td>
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<tr>
<td>611</td>
<td>Ia</td>
<td>1</td>
<td>+7q31-q33, +8, +12</td>
</tr>
<tr>
<td>613</td>
<td>Ille</td>
<td>2</td>
<td>+1q23-qter, +2q32-q35, -3p12-p14, +3q21-qter/3q22-qter, +5p14-q13, +8q13-qter/8q22-qter, +18q11.2-q21</td>
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<tr>
<td>616</td>
<td>Ille</td>
<td>2</td>
<td>+1p10-q24, +2p23-q21, -3p21-qter, +3q, -4p10-q28, +5p, -5q13-q21, -X, +6p10-q21, -6q22-qter, +7p12-qter, -8p, +8q8q22-qter, -9q22-qter, -10q21-qter, +11q21-qter, +12p11-q13, +13q21-qter, -18q21-qter, +19, +20q, -21</td>
</tr>
<tr>
<td>621</td>
<td>Ilb</td>
<td>1</td>
<td>+3q25-qter</td>
</tr>
<tr>
<td>666</td>
<td>III</td>
<td>2</td>
<td>+1qcen-q31, +3q24-qter, -Xp21-p1ter, +6q24-qter, -7p21-p1ter, +7q31-qter, -8p, +8q8q21.1-qter, +9q13-q22, +11q13-q23</td>
</tr>
<tr>
<td>676</td>
<td>III</td>
<td>3</td>
<td>+5p13-qter, +6p21.1-p1ter, +8q12-qter, +17q23-qter, +20q</td>
</tr>
<tr>
<td>787</td>
<td>Ic</td>
<td>3</td>
<td>+3q25-qter, +6q21.1-qter, -4q12-qter, +11q21-qter, -12q12-qter, +20q</td>
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<td>IV</td>
<td>3</td>
<td>+3q23-q4, +8q21.1-qter, -18</td>
</tr>
<tr>
<td>855</td>
<td>Ille</td>
<td>3</td>
<td>+1p32-q34.3, +1q, +3q, +Xp14-q24, +8q21.3-qter, +12p, +16p13.1-p1ter</td>
</tr>
<tr>
<td>1027</td>
<td>Ile</td>
<td>2</td>
<td>+3q22-qter, -4q13-qter, -5q13-q23, +Xq, -6q24-qter, -9q, -11p12-pter, +11q12-q13, +12p-pter, -13q, +20p1te-q13.2</td>
</tr>
</tbody>
</table>

*+, gains; –, losses. High-level amplifications are in bold.

(70%) and 8q (75%), with minimum common regions 3q25-qter and 8q22-qter, respectively. The other regions of frequent gains were 1q (40%), 5p (30%), 7q (35%), 12p (40%), and 20q (30%). High-level amplifications were found in six tumors, and the minimum common regions were 3q25-q28 (three cases), 8q22-qter (four cases), and 12p (one case). The most frequent regions of loss were 18q (35%), 8p (30%), 5q (30%), and 4q (25%).

In stage I and II (n = 5) and stage III and IV (n = 15) carcinomas, the mean numbers of DNA copy number changes/sample were 4.0 and 9.9, respectively. All of the high-level amplifications were detected in stage...
III carcinomas. Comparing the tumor grades, the mean numbers of changes were 5.0 (grade 1), 11.4 (grade 2) and 7.0 (grade 3), respectively.

The CGH results of serous uterine and serous ovarian carcinomas have been described in detail (7, 9). Comparison of the CGH results in serous carcinomas of the fallopian tube, uterus, and ovary revealed remarkable similarity of genomic alterations (Fig. 1).

Discussion

Our CGH study on primary fallopian tube carcinomas disclosed extensive genomic alterations and no normal karyotypes in any of the tumors. Karyotypic analysis of one case (12) and no LOH studies of fallopian tube carcinoma have been reported thus far. Hence, the genomic imbalances observed in this study are novel for this tumor type.

In fallopian tube carcinoma, the most common regions of gains and amplifications were at 3q and 8q, i.e., the same regions as in serous carcinomas of the uterus and ovary (7, 9, 13, 14). At 3q amplicon, telomerase RNA gene (3q26.3) is the only gene reported to be amplified in ovarian carcinoma (15). The large size of the commonly gained segment suggests that additional oncogenes important in serous gynecological carcinomas reside at distal 3q. CMYC at 8q24.1, known to be amplified in ovarian carcinoma (16), is located in the amplicon we detected. The common region of overrepresentation encompassed also a more proximal region (8q22-q23), implying the localization of other significant oncogenes at 8q.

In fallopian tube carcinoma, losses were less frequent than gains, and the most common sites of underrepresentation were 8p and 18q. These findings are similar to the previous observations on serous uterine (7) and ovarian carcinomas (9, 13, 14). The presence of LOH at 8p and 18q in the serous fallopian tube and uterine carcinomas is known; but, in ovarian carcinomas frequent LOH at these sites has been detected (17), supporting the hypothesis of tumor suppressor gene loci here. The deletion unit we observed at 18q21.1-qter includes two well characterized tumor suppressor genes, DCC and DPC4, but the minimal common region of allelic loss in ovarian carcinoma is outside the DCC and DPC4 loci (18), suggesting the presence of additional tumor suppressor genes in this region.

Molecular genetic studies, particularly on colorectal tumors, have provided evidence for the progressive and accumulative nature of the genetic defects underlying tumorigenesis. The ability of CGH to give information on the entire genomic profile of tumors in one analysis makes it a useful tool in studying the molecular genetic pathogenesis of tumors and screening for the chromosomal regions harboring potentially important oncogenes and tumor suppressor genes. The commonly gained regions detected in this study encompass about 8%, and the deleted regions encompass about 4% of the whole genome, respectively. The high number and the extent of changes may partially reflect a general genomic instability, especially in the high-grade tumors. Targeted expression studies, allelic and mutation analyses, are needed to narrow down the candidate regions detected by CGH screening and to identify the individual genes that play a role in serous carcinoma of the reproductive tract.

In organogenesis of the mammals, Müllerian ducts give rise to the surface epithelium of the ovary, the fallopian tubes, the uterus, the cervix, and the upper part of the vagina (19). In adult women, the epithelium of all these organs has the potential of developing serous, endometrioid and mucinous tumors resembling epithelium of the fallopian tube, uterine cavity, and endocervix, respectively. In histopathological examination, serous carcinomas of the fallopian tube, uterus, and ovary are indistinguishable. They also have the propensity of invasive behavior, early dissemination through peritoneal cavity, and poor overall prognosis (20). P53 mutation is highly prevalent in all these carcinomas (4, 5, 11, 21). By CGH, all of the three serous carcinomas showed frequent and extensive genomic imbalances with gains predominating over losses. Also the regions of the most common copy number changes were nearly identical. It is of note that the pattern of genomic alterations is distinct from that found in other histological subtypes of uterine and ovarian carcinomas (7, 8). The present and previous (7–9, 21) results emphasize histology as an important determinant in gynecological carcinomas. In particular, serous carcinomas of the Müllerian derivatives seem to represent a distinct entity irrespective of the site.

References

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