Histogenetic Analysis of Ovarian Germ Cell Tumors by DNA Fingerprinting

Masaki Inoue,1 Masami Fujita, Chihiro Azuma, Fumitaka Saji, and Osamu Tanizawa

Department of Obstetrics and Gynecology, Osaka University Medical School, Osaka 553, Japan

ABSTRACT

The histogenesis of ovarian germ cell tumors (11 mature teratomas, three malignant transformations of mature teratomas, two immature teratomas, and four dysgerminomas) was investigated genetically using minisatellite DNA probes 33.15 and 33.6 for person-specific restriction fragment length polymorphism (DNA fingerprint) analysis. The DNA fingerprints of six ovarian teratomas were identical with those of mononuclear cells from each host, while some polymorphic bands observed in the host mononuclear cells were lost in the DNA fingerprints of the other five cases. The cases of malignant transformation of mature teratoma and immature teratoma showed that some polymorphic bands of DNA fingerprints from the host mononuclear cells were absent in the tumor tissues. In four cases with dysgerminomas, the DNA fingerprints of tumors were completely identical with those of the respective host mononuclear cells. The present results suggest that mature cystic teratomas of the ovary arise from germ cells arrested at various stages of meiosis, while immature teratomas are derived from postmeiotic germ cells. Malignant transformation may occur exclusively in the mature teratomas arising from postmeiotic germ cells. Dysgerminomas develop from premeiotic oogonia (primordial germ cells). Thus, DNA fingerprints are a useful and sensitive tool for identifying the pathogenesis of germ cell tumors.

INTRODUCTION

Germ cell tumors embrace morphologically heterogeneous neoplasms considered to be originated from germ cells of the gonad and are the most common ovarian tumors in women during the second and third decades of life, accounting for approximately 20% of all ovarian tumors (1). However, the histogenesis of these tumors is unclear and has been a matter of speculation and dispute for decades. Much of the early work on the histogenesis of ovarian germ cell tumors was done by Tei- lum (2, 3), who proposed that such neoplasias originate from primitive germ cells and could give rise to either dysgerminoma or tumor of totipotential cells; the latter could then differentiate into embryonal carcinoma, which in turn could form neoplasms of extraembryonic structures (yolk sac tumors and choriosarcomas), or embryonic structures (teratomas). These views based on histopathological studies were further supported by the embryological studies of Witschi (4) and Gillman (5) and later by the experimental work of Stevens (6) and Pierce et al. (7, 8) on germ cell tumors in rodents. In recent times, two main theories for the histogenesis of teratomas have been proposed: an origin from segregated blas-tomes at an early stage of embryonic development and an origin from a parthenogenetic primordial germ cell. The latter theory has been steadily gaining support in clinicopathological studies, animal experiments, and cytogenetic studies and is now generally accepted (9, 10). Linder and coworkers (11–13) demonstrated that benign ovarian teratomas arise from a single germ cell after the first meiotic division using both cytogenetic techniques and electrophoretic variants of enzyme markers. On the other hand, Carritt et al. (14) observed a heterozygous pattern of chromosomal heteromorphism in four ovarian teratomas and proposed that ovarian teratomas might originate from a germ cell prior to the first meiosis. This speculation was further supported by Zeuthen et al. (15) who observed centrometric heterozygosity in one ovarian teratocarcinoma cell line. Recently, however, some investigators (16, 17) observed both heterozygosity and homozygosity of chromosome and enzyme markers in several ovarian tumors and postulated that ovarian teratomas arise from germ cells in a number of different ways. Thus, the pathogenesis of ovarian teratomas has not been completely clarified by conventional cytogenetic methods and remains to be investigated by more sensitive and specific techniques.

Recent advances in molecular technology permit the characterization of genetic variations at the DNA level. One highly sensitive and discriminating method of analysis is based upon DNA polymorphisms, which can be detected as RFLPs2 by a probe based on a tandem-repeat of the core sequence (18). One such probe, the minisatellite DNA probe described by Jeffreys et al. (19, 20) can detect many regions of great variability within the human genome and can provide an individual-specific DNA “fingerprint.”

We have reported a preliminary observation on the histogenesis of ovarian teratomas using a minisatellite DNA probe, 33.15, and suggested that mature cystic teratomas of the ovary arise from germ cells before Meiosis I, while immature teratomas are derived from germ cells after Meiosis I (21). In the present study, a larger number of cystic teratomas, their malignant transformations, immature teratomas, and dysgerminomas of the ovary were analyzed to determine the origin of such types of ovarian germ cell tumors using minisatellite DNA probes 33.15 and 33.6.

MATERIALS AND METHODS

Patients. Samples were obtained from 20 patients with ovarian tumors who underwent laparotomy at the Department of Obstetrics and Gynecology, Osaka University Medical School. Histological diagnosis was carried out according to the WHO histological typing system (22). The clinicopathological data are listed in Table 1.

We removed a small piece of tissue from the center of each tumor, taking care not to include any material from the ovarian capsule, and washed it repeatedly in cold phosphate-buffered saline, pH 7.2, to exclude contamination by the blood of the host. Mononuclear cells were also obtained from the peripheral blood of the patients for DNA analysis.

DNA Analysis. High-molecular-weight DNA was extracted from the tumor tissues and mononuclear cells of the patients as described elsewhere (23). Four μg of each DNA sample was digested with endonuclease HindII at 37°C for 3 h, electrophoresed on 0.7% agarose gel, and then transferred by blotting to nitrocellulose filters according to the method of Southern (24). After baking the filters under a vacuum at 80°C for 3 h, a 32P-labeled minisatellite DNA probe was hybridized at 62°C for 12 h in Denhardt's solution, 1 μl NaCl, 50 μm Tris-HCl (pH 7.4), 0.1% sodium dodecyl sulfate, 10 μg EDTA, and 0.1 mg/ml of denatured sonicated salmon sperm DNA. The filters were then

Received 5/21/92; accepted 9/30/92.

2 The abbreviation used is: RFLP, restriction fragment length polymorphism.
DNA FINGERPRINT OF OVARIAN GERM CELL TUMORS

Table 1: Clinicopathological findings and DNA fingerprintings of germ cell tumors of the ovary

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>P/Ga</th>
<th>Diagnosis</th>
<th>DNA fingerprinting (pre- and post-Meiosis I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>1/3</td>
<td>Mature teratoma</td>
<td>Pre</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>0/0</td>
<td>Mature teratoma</td>
<td>Pre</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>2/3</td>
<td>Mature teratoma</td>
<td>Pre</td>
</tr>
<tr>
<td>4</td>
<td>68</td>
<td>4/4</td>
<td>Mature teratoma</td>
<td>Pre</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>2/3</td>
<td>Mature teratoma</td>
<td>Pre</td>
</tr>
<tr>
<td>6</td>
<td>17</td>
<td>0/0</td>
<td>Mature teratoma</td>
<td>Pre</td>
</tr>
<tr>
<td>7</td>
<td>26</td>
<td>1/1</td>
<td>Mature teratoma</td>
<td>Post</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>0/0</td>
<td>Mature teratoma</td>
<td>Post</td>
</tr>
<tr>
<td>9</td>
<td>28</td>
<td>2/2</td>
<td>Mature teratoma</td>
<td>Post</td>
</tr>
<tr>
<td>10</td>
<td>21</td>
<td>0/0</td>
<td>Mature teratoma</td>
<td>Post</td>
</tr>
<tr>
<td>11</td>
<td>28</td>
<td>2/3</td>
<td>Mature teratoma</td>
<td>Post</td>
</tr>
<tr>
<td>12</td>
<td>70</td>
<td>5/6</td>
<td>Malignant transformation of a mature teratoma</td>
<td>Post</td>
</tr>
<tr>
<td>13</td>
<td>56</td>
<td>2/4</td>
<td>Malignant transformation of a mature teratoma</td>
<td>Post</td>
</tr>
<tr>
<td>14</td>
<td>64</td>
<td>6/3</td>
<td>Malignant transformation of a mature teratoma</td>
<td>Post</td>
</tr>
<tr>
<td>15</td>
<td>18</td>
<td>0/0</td>
<td>Immature teratoma</td>
<td>Post</td>
</tr>
<tr>
<td>16</td>
<td>21</td>
<td>0/0</td>
<td>Immature teratoma</td>
<td>Post</td>
</tr>
<tr>
<td>17</td>
<td>19</td>
<td>0/0</td>
<td>Dysergerminoma</td>
<td>Pre</td>
</tr>
<tr>
<td>18</td>
<td>16</td>
<td>0/0</td>
<td>Dysergerminoma</td>
<td>Pre</td>
</tr>
<tr>
<td>19</td>
<td>24</td>
<td>0/0</td>
<td>Dysergerminoma</td>
<td>Pre</td>
</tr>
<tr>
<td>20</td>
<td>22</td>
<td>0/0</td>
<td>Dysergerminoma</td>
<td>Pre</td>
</tr>
</tbody>
</table>

a P/G, parity (the number of pregnancies reaching viability)/gravida (the number of pregnancies irrespective of their outcome).

Table 1 summarizes the conclusions based on the results of the present study using minisatellite DNA probes 33.15 and 33.6. In the course of development, the primitive germ cells undergo two special divisions, Meiosis I and Meiosis II. Just before Meiosis I, the primitive germ cells replicate their DNA and contain 46 chromosomes, each of which is a double structure. After Meiosis I, the number of chromosomes is reduced to half the number of a somatic cell (23 double-structured chromosomes), while the amount of DNA equals that of a somatic cell. In Meiosis II, the 23 double-structured chromosomes are divided to single structures, at which point the amount of DNA is half that of a somatic cell. Consequently, a finding that some polymorphic bands are deleted in the tumor DNA may suggest that the tumor arose from a postmeiotic stage germ cell. Conversely, a finding that the tumor and the host have the same band patterns could indicate that the tumor arose from a premeiotic germ cell. Thus, the present results suggest that mature cystic teratomas of the ovary arise from germ cells arrested at various stages of meiosis, while immature teratomas are derived from germ cells after Meiosis I. The malignant transformation may take place in the mature teratomas derived from germ cells after Meiosis I. Dysgerminomas arise from premeiotic germ cells.

RESULTS

Fig. 1 shows the representative profiles of the RFLP band patterns detected by minisatellite DNA probes 33.15 and 33.6 in six sets of the ovarian germ cell tumor tissues and the peripheral mononuclear cells of the respective host. In the 4- to 20-kilobase region, each patient displays about 10 to 20 polymorphic bands, constituting person-specific RFLPs called DNA fingerprints, while fragments smaller than 4 kilobases do not show person-specific polymorphisms (20). In the six cases (Cases 1 to 6) of 11 mature teratomas (dermoid cysts), each DNA fingerprint was identical to that of the mononuclear cells from the host. In the other five cases, some polymorphic bands observed in the host were deleted from the DNA fingerprints of the tumor tissue. It is very interesting that all three cases of malignant transformation from a dermoid cyst showed fewer polymorphic bands than the host mononuclear cells. Two immature teratomas also showed that some polymorphic bands observed in the host were deleted from the DNA fingerprints of the tumor tissue. In all four dysgerminomas, the RFLP band patterns of the tumor tissues were identical with those of each host.

DISCUSSION

Early genetic studies of germ cell tumors relied heavily on analysis of sex chromatin (Barr bodies) and were complicated...
by inaccurate observations and an inability to predict the genotype from the nuclear sex (10). Karyotypic analysis of benign cystic teratomas of the ovary soon revealed a diploid 46XX genotype in most benign lesions (26, 27). Using the analysis of electrophoretic variants of enzyme markers, Linder and Power (11, 12) demonstrated some heterozygous host alleles distant from the centromere in some ovarian teratomas, although most teratomas arising in 31 heterozygous hosts were homozygous. Chromosome-banding heteromorphisms located near the centromere have also been shown to be homozygous in most ovarian teratomas (13, 28). Therefore, it has been speculated that teratomas are derived from post-Meiosis I cells and that heterozygous teratomas arise from cross-over between the centromere and the relevant locus (11-13, 28).

Although most ovarian benign teratomas are postmeiotic, several cases with heterozygous centrometric banding heteromorphisms have been reported, suggesting that some teratomas may arise from a premeiotic germ cell. Parrington et al. (29) found that 8 of 12 teratomas arising in 10 patients displayed heterozygous centrometric heteromorphism identical to that of the host. Six of these 8 teratomas developed in 3 patients with multiple teratomatosis. Nomura et al. (30) also applied chromosome banding, human leukocyte antigen determination, and enzyme analysis to 16 teratomas from 4 patients and found that 3 teratomas in 2 patients had heterozygous centrometric markers identical to those of the host. A triploid mature ovarian teratoma arising in a diploid host was recently described, suggesting another mechanism by which a haploid polar body may be fused with a diploid ovum (31). Thus, the results obtained by chromosome analysis have supported the speculation that benign ovarian teratomas arise from germ cells in a number of different ways.

In the present study, we introduced a newly developed method of DNA fingerprinting for histogenetic analysis of ovarian teratomas. The probes 33.15 and 33.6 used in the present study can simultaneously detect highly variable regions widely dispersed in the human genome and can provide individual-specific RFLPs called DNA fingerprints (18, 19). In view of the fact that these polymorphism fragments are inherited from host cells in a Mendelian fashion, DNA fingerprint analysis is very useful in many genetic investigations, such as zygosity determination in multiple pregnancy, mapping of human linkage groups, indirect determinations of genetic disease loci, and detection of meiotic recombinations (25, 32). Along these lines, we previously reported a new application of minisatellite DNA probe 33.15 to the genetic analysis of complete hydatidiform moles and demonstrated the value of this probe for the verification of androgenesis as the cause of complete moles (33).

The germ cells are well known to undergo two special divisions, Meiosis I and Meiosis II, by which the number of chromosomes and then the amount of DNA are reduced to half those of a somatic cell, respectively. Deletion of several polymorphic bands in a tumor tissue could suggest that the tumor arose from a postmeiotic stage germ cell. As shown in Fig. 1, the DNA fingerprints of six ovarian teratoma tissue specimens were identical with those of the respective host mononuclear cells, but some differences between the DNA fingerprints of the tumors and their respective hosts were observed in the other five cases. This was consistent with the difference in DNA fingerprints between complete hydatidiform moles and the male consort. These results suggest that benign mature ovarian teratomas arise from germ cells arrested at various stages of meiosis, which is in agreement with the previous results obtained by chromosome analysis (29-31). On the other hand, two immature teratomas showed fewer bands of polymorphic fragments than those of their host mononuclear cells, suggesting that immature teratomas arise from postmeiotic germ cells. A few new bands seem to have appeared in tumor tissue of Case 15 when compared with the host mononuclear cells. However, these bands are also observed in the lane of the host, even though they are very weak. Such a difference in the intensity of bands between tumor and host DNA samples could be caused by a difference in the degree of DNA methylation, which could affect the efficacy of HindIII digestion. As another possible explanation, there may be rearrangement or mutation of tumor DNA in the chromosomes regions adjacent to the hypervariable tandem-repeat intron arrays which are detected by these probes (19, 34).

It is interesting that the bandings of DNA fingerprints from tumor tissues were fewer in number than those of the respective host in all three cases with malignant transformation of a mature teratoma, indicating that malignant transformation may occur exclusively in mature teratomas arising from postmeiotic germ cells. Since such tumors arising from the postmeiotic germ cells have fewer chromosomes than the host, they may easily and often transform to a malignant phenotype, and it may be that during that process the deletion of a tumor suppressor gene takes place. Actually, we have detected the overexpression and point-mutation of the tumor suppressor gene, p53, in such tumors.

Histogenetic analysis of dysgerminoma has been less common, compared to teratomas. The morphological similarity between dysgerminoma cells and germ cells of the developing ovary strongly suggests that these tumor cells are of germ cell origin. Although some investigators (35) reported that the cells of some dysgerminomas have sex chromatin bodies, Asadourian and Taylor (36) found that the tumor cells of 22 dysgerminomas were sex-chromatin negative, while the stromal cells were sex-chromatin positive. They also showed that the DNA content in the nuclei of dysgerminoma cells is approximately double the content of the control somatic cells. All of these findings point toward an origin from primordial germ cells at the beginning of the first meiotic division. The present study using DNA fingerprints supports such a view. The recently developed method using the specific probes, 33.15 and/or 33.6, definitely provides greater accuracy than the conventional genetic tests and offers a useful and sensitive tool for the cytogenetic analysis of germ cell tumors.

ACKNOWLEDGMENTS

The authors wish to thank Masahiko Hori and Kiyofumi Kamiyama of Teijin Bio Laboratories, Inc., Tokyo, Japan, for their excellent technical assistance.

REFERENCES


DNA FINGERPRINT OF OVARIAN GERM CELL TUMORS


Histogenetic Analysis of Ovarian Germ Cell Tumors by DNA Fingerprinting

Masaki Inoue, Masami Fujita, Chihiro Azuma, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/52/24/6823

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.