Genetic Analysis of Ovarian Germ Cell Tumors by Comparative Genomic Hybridization

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ABSTRACT

Ovarian germ cell tumors (OGCTs) show a heterogeneity that is not seen in their testicular counterparts and include benign mature cystic teratomas, intermediate immature teratomas, malignant germ cell tumors (GCTs [dysgerminomas, endodermal sinus tumors, and mixed GCTs]), and GCTs arising in dysgenetic gonads of 46,XY individuals. Comparative genomic hybridization was used to analyze 27 OGCTs for regions of relative gain or loss. The analysis of 21 malignant OGCTs (12 dysgerminomas, 6 endodermal sinus tumors, and 3 mixed GCTs) demonstrated genetic alterations similar to those reported in adult testicular GCTs. The most common regions gained include chromosomes 12p (16 of 21 tumors), 21 (10 of 21 tumors), 8 (8 of 21 tumors), and 1q (6 of 21 tumors). The most common region lost was chromosome 13. Regions of high-level gain were identified at 12p11-12 and 4q11. The profile of gains and losses was similar in the different histological subtypes within this category. One tumor presented in a 46,XY patient; this tumor was diploid and showed a gain of 12p. Immature teratomas (six cases) showed only one case with an abnormality, which was a gain of chromosome 14. We conclude that malignant OGCTs are genetically similar to those found in the adult testis; however, immature teratomas show no consistent gains or losses and are therefore different from those presenting in the adult testis. A review of the literature suggests that genetic abnormalities in this group may herald a worse prognosis. Lastly, OGCTs in dysgenetic gonads arise in a diploid rather than a tetraploid cell line, yet they also show a gain of 12p.

INTRODUCTION

GCTs are a diverse group of neoplasms that may arise at any age from infancy to late adulthood. GCTs are encountered in both sexes in gonadal as well as extragonadal sites and have been histologically classified into several subtypes. These include mature and immature teratoma, germinoma (dysgerminoma and seminoma), EST (yolk sac tumor), embryonal carcinoma, and choriocarcinoma. Independent of these histological groups, five categories have emerged based on patient age, tumor site, and clinical behavior:

(a) The largest and most widely studied category is composed of tumors arising in the testis of males 15-34 years of age. Tumors in this category are aneuploid, and mathematical analysis of DNA content has suggested that these tumors arise in a tetraploid precursor stem line with subsequent nonrandom chromosomal loss (1, 2). Approximately 80% of these show a characteristic cytogenetic abnormality, the isochromosome 12p (i(12p)), regardless of histological subtype (3-7). The important gene or genes lost on 12q and gained on 12p have not been identified.

(b) Testicular GCTs show a biphasic age distribution, and the second GCT category is composed of testicular tumors of early childhood (1-4 years). The vast majority of these show EST histology, and genetic analyses demonstrate deletion of 1p and 6q and do not show the i(12p) (8-11). Rare tumors in this age group show only teratoma histology; these are benign, diploid, cytogenetically normal neoplasms (12, 13).

(c) Extragonadal GCTs are also biphasic; those that arise in early childhood comprise the third GCT category. These are most frequently large, benign teratomas occurring in the sacrococcygeal region of infants. Less frequently, malignant EST histology may be present. Genetic analyses of extragonadal teratomas and ESTs in young children show no difference from those occurring in the early childhood testis (9, 11, 13).

(d) Extragonadal GCTs in older children and adults most commonly arise in the mediastinum and brain and comprise the rarest subset of GCTs. Only a few of these tumors have been cytogenetically analyzed; some malignant tumors show the i(12p) (14-16).

(e) The final group, the OGCTs, forms the basis for our study. OGCTs constitute 20% of ovarian neoplasms in all age groups, are most frequent in adolescence and young adulthood, and show the full spectrum of histological subtypes. However, OGCTs show a biological heterogeneity that is not seen in the testis and can be subdivided into four subgroups based on histology and clinical behavior. These include benign mature teratomas, intermediate immature teratomas, malignant GCTs (including dysgerminomas, ESTs, and mixed GCTs), and lesions arising in phenotypic females with 46,XY gonadal dysgenesis. Few cytogenetic studies of malignant OGCTs have been performed. Although the genetic relationships among the OGCT subgroups and between OGCT and other GCT categories have not been sufficiently explored, OGCTs are treated with regimens similar to those of their testicular counterparts. The pathogenesis of subgroups of these neoplasms may differ from that of testicular GCTs; theoretically, these may respond to different therapeutic regimens.

Classic cytogenetic analysis remains the best method for screening for genetic abnormalities. However, these rare lesions are often not submitted for tissue culture or do not grow well in culture and yield few mitoses for analysis. CGH is a technique that enables screening of the entire genome for genetic gains and losses and does not require tumor growth in culture (17, 18). We report the analysis of 27 OGCTs by CGH.

MATERIALS AND METHODS

Patient Population. Frozen tissue of 27 OGCTs was obtained from 25 patients treated at The Johns Hopkins Hospital or by Pediatric Oncology Group-affiliated institutions or from the Cooperative Human Tissue Network. Tumors were evaluated for histological pattern according to the WHO classification (19) and include 12 dysgerminomas from 10 patients, 6 immature teratomas, 6 ESTs, and 3 tumors showing multiple malignant histological types (mixed GCTs).

DNA Extraction. Each frozen tumor was embedded in optimal cutting temperature medium, and a histological section was prepared and examined microscopically. Regions of necrosis and nontumor regions were dissected off the block. If at least 70% viable tumor cellularity was present in the remaining tissue, DNA extraction was performed using standard techniques (20). Normal control DNA was similarly prepared from the lymphocytes of a healthy male donor.

Labeling of Tumor and Reference DNA. DNA (1 μg) was labeled using DNA polymerase 1 and DNase, labeled dUTP (fluorescein-12-dUTP for tumor...
DNA and Texas Red-5-dUTP for reference DNA), dATP, dGTP, dCTP (20 μM), Tris (50 mM), MgCl₂ (5 mM), mercaptoethanol (10 mM), and BSA (10 μg/ml) at 15°C. The amount of DNase and polymerase and the reaction time were adjusted to achieve DNA fragment lengths of 500-2000 bp, as determined on a 1% agarose gel.

Cytogenetics. Cot-1 DNA (20 μg; to block binding to repetitive DNA sequences) was combined with 300 ng of FITC-labeled tumor DNA and 300 ng of Texas Red-labeled reference DNA and coprecipitated. The pellet was resuspended in hybridization media (50% formamide, 10% dextran sulfate, and 2× SSC). The resulting probe was denatured at 75°C for 10 min and subsequently partially reannealed at 37°C for 30 min. The probe was hybridized to methotrexate-synchronized reference metaphases on glass slides, pre-aged 5 days, pretreated with 1% NP40 in 2× SSC for 30 min at 37°C, and denatured at 72°C in 70% formamide and 2× SSC. The slides were placed in a moist 37°C chamber for 3 days. After washing for 5 min each in 2× SSC at 72°C, 2× SSC at 37°C, and water at room temperature, the slides were counterstained with 0.1 μg/ml 4',6-diamidino-2-phenylindole in antifade.

Microscopy and Analysis. Gray level images were acquired for each fluorescent dye with a charge-coupled device camera on a Zeiss Axioscope epifluorescence microscope using the Applied Imaging Corporation’s dedicated Cytovision software and hardware. Chromosomes were identified using reverse 4',6-diamidino-2-phenylindole banding. The background fluorescence was subtracted, and the green:red ratio of each entire metaphase was normalized to 1.0. Data from at least 12 representatives of each chromosome were combined to generate an average ratio profile and SE for each chromosome. Upper and lower thresholds of >1.2 and <0.8 were used to interpret the gain or loss of chromosomal material (21). DNA from healthy tonsillar tissue and other translocations. Four tumors showed a gain of the entire short arm of chromosome 12 (Fig. 2C). Isochromosome formation of the short arm of chromosome 12, reported in testicular GCTs, would result in this appearance; however, 12p gain could also result from other translocations. Four tumors showed a gain of the entire chromosome 12. Two cases showed a restricted region of high-level gain at 12p11–12 (Fig. 2A). Other common regions of gain were chromosomes 8 and 21 (8 of 19 patients with malignant OGCT each), the long arm of chromosome 1 (6 of 19 patients), and chromosome 7 (5 of 19 patients). Losses were less frequent; the most common region of loss was chromosome 13 (5 of 19 patients), with 1 tumor showing loss confined to 13q21. Other than the gain of 12p, only one tumor showed a small region of high-level gain characteristic of true gene amplification; tumor 6 showed a high-level gain at 4q11 (Fig. 2B).

Three dysgerminomas merit further attention. Patient 3 presented with a stage I ovarian dysgerminoma. Two years later, she presented with and died of disseminated mast cell disease, which by cytogenetic analysis showed 48,XX,+3,i(12)(p10),+mar. Patient 9 presented at the age of 11 years with primary amenorrhea. Evaluation revealed normal female external genitalia, a 46,XY constitutional genotype, and a right ovarian mass that was a dysgerminoma. The left ovary showed a small gonadoblastoma, which was not able to be analyzed. Patient 10 presented with a large right ovarian mass (greatest dimension, 24 cm; tumor 10a) and a smaller left ovarian mass (greatest dimension, 8 cm; tumor 10b). Staging performed at the time of the initial surgery showed no lymph node metastasis. A second-look surgery performed 3 months later revealed metastatic tumors in four lymph nodes and on the diaphragmatic surface (tumor 10c).

Within the malignant OGCT category, there was no correlation between the histological subtype, patient age, and regions of chromosomal gain and loss as listed in Table 1. The genetic changes identified in the malignant OGCTs are represented schematically in Fig. 1. Representative profiles from individual cases are shown in Fig. 2.

Malignant OGCTs. The most common change identified was a gain of 12p, which was seen in 14 of 19 (74%) patients with malignant OGCTs. In eight cases, the profiles showed a gain of the entire short arm of chromosome 12 (Fig. 2C). Isochromosome formation of the short arm of chromosome 12, reported in testicular GCTs, would result in this appearance; however, 12p gain could also result from other translocations. Four tumors showed a gain of the entire chromosome 12. Two cases showed a restricted region of high-level gain at 12p11–12 (Fig. 2A). Other common regions of gain were chromosomes 8 and 21 (8 of 19 patients with malignant OGCT each), the long arm of chromosome 1 (6 of 19 patients), and chromosome 7 (5 of 19 patients). Losses were less frequent; the most common region of loss was chromosome 13 (5 of 19 patients), with 1 tumor showing loss confined to 13q21. Other than the gain of 12p, only one tumor showed a small region of high-level gain characteristic of true gene amplification; tumor 6 showed a high-level gain at 4q11 (Fig. 2B).

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Fig. 1. Schematic diagram of the gains and losses identified in 17 patients with 19 malignant OGCTs. Lines to the left and right of the chromosomes indicate the regions lost and gained, respectively. Thick bars represent regions of high-level gain.

between the pattern of gains and losses and the histological subtype. One possible exception was the presence of a deletion of distal 1p identified in two ESTs but not identified in other subtypes. Two ESTs showed no abnormalities.

Immature Teratomas. Of the six immature teratomas analyzed, five were grade 2, and one showed grade 3 histology. The immature teratomas clearly differed from the malignant OGCTs in that they showed none of the abnormalities listed above. In fact, of the six immature teratomas analyzed, only one numerical abnormality was noted, a gain of chromosome 14 (patient 24).

Cytogenetic Analysis. Fresh tissue was available for tissue culture for seven tumors. Five tumors were able to be fully analyzed, and the karyotypes were concordant with the CGH findings. One tumor showed a normal karyotype but showed profile deviations by CGH. This may reflect the failure of tumor cell growth in tissue culture. Patient 10 had three tumors submitted for cytotgenetic analysis. The dominant and presumably primary tumor showed i(6p), i(12p), +21, and a marker chromosome that was most consistent with der(5)t(5;?)(q3.;?) and therefore contained all of chromosome 5 except for the telomeric regions. The lack of a clear loss of chromosome 5 by CGH would support this. In our experience, the loss of one of four copies of a chromosome within a tetraploid sample is often not detectable with confidence by CGH. The smaller contralateral ovarian mass and the subsequent metastasis of this patient were analyzed, although few metaphases that were of low quality were available. However, both clearly contained the marker chromosome seen in the dominant tumor and also showed chromosomes consistent with an i(12p) and i(6p).

DISCUSSION

GCTs are a unique group of tumors that arise from the primordial germ cell. Despite this common cell of origin, the resulting tumors vary in age and site of presentation, in histological appearance, and in the sex of the patient. Studies of these different tumors may increase our understanding of normal germ cell development within these different environments. In addition, GCTs are able to show a broad range of spontaneous and induced differentiation. Cell lines derived from these lesions constitute an important vehicle for studying neoplasia and differentiation. Despite this, the current level of knowledge of the genetic and epigenetic factors driving these tumors is rudimen-
tary. Whereas GCTs of the young adult testis have been widely studied, those arising in the ovary and those arising in infants have received remarkably little attention. It is the goal of this study to provide a genetic analysis of ovarian immature teratomas and malignant GCTs. Current cooperative groups have made these multi-institutional studies possible.

The most common OGCT is the mature cystic teratoma. Cytogenetic analyses of over 300 mature ovarian teratomas have shown abnormalities in less than 5% of tumors, none of which are consistent or recurrent (23–26). Molecular analyses have suggested that most mature ovarian teratomas arise due to errors in meiosis (23, 27). Much less frequent are immature ovarian teratomas, which have no true testicular counterpart. The few immature ovarian teratomas that have been analyzed cyogenetically show a higher frequency of numerical abnormalities than do mature teratomas, but without any consistent abnormalities (28–33). Table 2 lists the clonal abnormalities previously reported in immature teratomas, the majority of which are grade 3. An additional three cases have been reported that show a normal karyotype; these cases were all grade 1 or 2. The six pure immature teratomas analyzed by CGH in this study demonstrated only a single case with a gain of chromosome 14; it is of interest that this tumor was the only grade 3 immature teratoma analyzed.

Ovarian immature teratomas are most common in adolescent females. Clinical decisions regarding therapy are complicated by the need to preserve fertility, the excellent prognosis in many tumors, and the ability of the tumors to metastasize or develop malignant histological components. Many current protocols seek to reduce chemotherapy exposure in these patients. Biological parameters that would predict which tumors will behave poorly would be useful; such efforts are extraordinarily difficult due to the rarity of these tumors, their commonly good prognosis, and inconsistently applied histological grading systems. Of the cyogenetically abnormal immature teratomas previously reported, all recurred multiple times despite chemotherapy, with the exception of a tumor showing a 47,XXX karyotype. In contrast, all of the previously reported karyotypically normal immature teratomas did not recur. This suggests the possibility that cyogenetically abnormal immature teratomas may have a worse prognosis. The clinical outcome of the majority of immature teratomas in the current study is not known. Studies of DNA content in ovarian immature teratomas have supported this trend. Silver et al. (13) reported the DNA content of five adolescent ovarian immature teratomas. Two grade 1–2 immature teratomas were diploid, whereas three grade 3 lesions were aneuploid. More recently, Baker et al. (34) studied nine adolescent immature teratomas (grades 1–3), all of which were diploid, and none of which recurred. Taken together, the prior and current cytogenetic and DNA content studies suggest that increasing aneuploidy is associated with a worse prognosis. However, for prognostic purposes, DNA content is not sufficiently sensitive to detect gains of one to two small chromosomes. It will be important for future cooperative protocols seeking to reduce chemotherapy dosage to examine the efficacy and cost of genetic analysis versus histological grading to guide therapy.

Malignant GCTs represent the third OGCT category. Dysgerminomas are the most common histological subtype, followed by ESTs and mixed GCTs. Classic cytogenetic analysis of only three ovarian dysgerminomas, three ESTs, and two mixed GCTs has been reported in the literature and is listed in Table 2 (33, 35–39). The current study contributes 19 additional cases. The most common abnormality identified was a gain of the short arm of chromosome 12, which was seen in 74% of the patients. There was a high incidence of 12p gain in all categories of OGCTs except immature teratomas. CGH analysis of testicular GCTs has demonstrated a similar high frequency of 12p gain, and in rare cases, 12p gain was specifically restricted to the 12p11.2-p12.1 region, suggesting the presence of a novel oncogene in this region (40, 41). The current study shows two OGCTs with a gain localized to 12p11–12. Other recurrent abnormalities seen in OGCTs were gain of the entire chromosome 21 (42% of malignant OGCTs), loss of chromosome 13 (26%), gain of chromosome 8 (42%), and gain of chromosome 1q (32%). Recent studies of testicular GCT by CGH have similarly shown recurrent loss of chromosome 13 (38%), gain of chromosome 21 (45%), gain of chromosome 1q (36%), and gain of chromosome 8 (45%; Refs. 40 and 41). High-level gain of the 4q11–12 region was identified in one dysgerminoma. Whereas this region is close to the centromere, a region more difficult to analyze by CGH, a deviation of this magnitude and reproducibility is not likely to be due to an artifact. Similar amplification has been reported in two adult testicular GCTs by CGH (41). Although the gene amplified in these tumors is not known, the genes for platelet-derived growth factor receptor α and KIT have been mapped to this region. A comparison of CGH analysis of ovarian malignant GCTs and ovarian epithelial neoplasms shows significant differences. The most common abnormalities identified in epithelial neoplasms include gain of 3q25–26, gain of 8q24, loss of 16q, and loss of 17pter–q21 (42). It is noteworthy, however, that 8 of 44 ovarian epithelial neoplasms showed 12p gain by CGH.

These data support the genetic similarity between ovarian and testicular adolescent and adult GCTs. This genetic similarity, coupled with previous reports that ovarian malignant GCTs arise in premeiotic germ cells (27), suggests that ovarian and testicular GCTs arise in a
primordial germ cell at a similar point in development. Normal ovarian cancer cells enter into the first phase of meiosis between 18–40 weeks gestation. Therefore, these data imply that ovarian malignant GCTs arise in utero, or that prolonged delay of entry into meiosis may be associated with carcinogenesis.

One case that we were fortunate to study was a dysgerminoma that was bilateral and subsequently metastatic (patient 10). This patient had a large right ovarian mass that by CGH showed a gain of 6p, 12p, and 21 and a smaller left ovarian mass and a later metastasis that both showed a gain of 7, 12p, and 21. Deviation of the 6p profile toward the right was present in these latter samples but did not meet the threshold for gain. Cytogenetic analysis of all three lesions was hindered by the low mitotic rate and poor morphology, but the same marker chromosome was identified in all three samples. Dysgerminomas, unlike other OGCT histological subtypes, are bilateral in 10% of patients. The cytogenetic and CGH results suggest that in this patient, the contralateral tumor represents a metastasis. The clinical outcome for the remaining GCTs is not known; therefore, it is not possible to correlate the gain or loss of chromosome 6 or 7 with clinical behavior.

Whereas the predominate pattern of gains and losses within malignant OGCTs was constant regardless of histological subtype, ESTs merit specific attention. ESTs are aggressive tumors that grow rapidly and metastasize early. Ovarian ESTs often arise multifocally within an immature teratoma, a phenomenon that is not seen in the adolescent and adult testis but is seen in infantile GCTs of the testis and extragonadal sites. This unique relationship between immature teratomas and ESTs has raised questions about possible genetic differences between ovarian ESTs and other malignant OGCT subtypes. Of the four prior ESTs cytogenetically analyzed, three contained the i(12p) (37–39, 43). The data we present suggest that ESTs do not substantially differ genetically from their other malignant counterparts. However, one intriguing finding was the presence of a deletion of distal 1p 1 seen in two ovarian ESTs and not in the other histological subtypes. Deletion of distal 1p has also been described in testicular and extragonadal ESTs of young children, tumors that do not show the i(12p) (8, 9, 11, 44). This potential genetic link between these two otherwise distinct groups of tumors needs further investigation.

An association between extragonadal GCTs and hematological malignancies is well established in the literature, with over 50 cases reported. The vast majority of the GCTs with this association are malignant mediastinal tumors in males (45–52). The associated hematological abnormalities include erythroleukemia, malignant histiocytosis, acute nonlymphocytic leukemia, myeloproliferative disorder, myelodysplasia, and recently, two cases with systemic mast cell disease. It has been proposed that the cells of the GCT may provide the stem line of the hematological malignancy. This is supported by the presence of the i(12p) in both the GCT and hematopoietic malignancy in several cases, including patient 3 in the current study (47, 51–53). No consistent abnormality distinguishes those GCTs who subsequently show hematological abnormalities. Only three OGCTs have been reported in association with hematological abnormalities, and two of these were 46,XY phenotypic females. It is of interest that the GCT of patient 3 arose in a diploid rather than an aneuploid cell line, similar to GCT arising in 46,XY phenotypic females (see below).

The final and rarest OGCTs are those that arise in phenotypic females with 46,XY gonadal dysgenesis. These patients often show a benign gonadal lesion, gonadoblastoma; in approximately 50% of patients with gonadoblastomas, a malignant GCT develops, most commonly dysgerminoma (54–56). This phenomenon is poorly understood; however, the tumors arising in dysgenetic gonads seem to differ biologically from those arising in normal gonads, in that they are diploid rather than aneuploid (34, 36). An i(12p) has been described in only one such tumor (36). The current study includes one patient with gonadal dysgenesis (patient 9). This dysgerminoma was analyzed for DNA content, which was diploid, and by classic cytogenetic analysis, which was 46,XY. Because of the likelihood that the normal metaphases were derived from nonneoplastic cells, fluorescence in situ hybridization was performed using a centromeric probe to chromosome 12 on interphase cells before tissue culture. This revealed only two signals of equal size per cell; tumors containing the i(12p) show signal numeric and size differences using these methodologies (4, 57). Insufficient tissue was available for further fluorescence in situ hybridization using 12p-specific probes or chromosome 12 paint probes. These findings suggest that whereas malignant GCTs arising in patients with gonadal dysgenesis do not require polyploidization, they seem to show a gain of 12p similar to that of their counterparts in normal females. Furthermore, in some tumors, gain of 12p may not correspond to the i(12p).

In summary, OGCTs can be separated into four distinct categories. Mature teratomas are diploid, karyotypically normal, benign lesions that arise due to errors in meiosis. Malignant OGCTs seem to be genetically similar to adult testicular GCTs, in that both groups demonstrate frequent 12p gain and a similar pattern of other gains and losses. Regions recurrently involved merit further study. Ovarian immature teratomas show no chromosome 12 abnormalities and no consistent gains or losses; therefore, they are different from those arising in the adult testis. Genetic abnormalities may herald a worse prognosis in these tumors. The transformation of an immature teratoma to an EST is associated with changes in DNA content, and the current study suggests that one genetic change in this transformation is the acquisition of an i(12p). Malignant OGCTs in dysgenetic gonads arise in a diploid cell rather than in a tetraploid cell, and the two tumors now reported show 12p gain.

REFERENCES


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