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Chromosome 12p abnormalities in dysgerminoma of the ovary: a FISH analysis

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Dysgerminoma is the most common malignant ovarian germ cell tumor and shares histological and immunophenotypical features with its testicular counterpart, seminoma. Chromosome 12p abnormalities are genetic hallmarks of testicular seminomas. Little is known about these genetic changes in dysgerminoma. We performed dual color fluorescence *in situ* hybridization (FISH) analyses with a centromeric α-satellite probe for chromosome 12 and a subtelomeric probe for 12p on paraffin-embedded tissue sections from 21 dysgerminomas and two gonadoblastomas. Chromosome 12p abnormalities were detected in 81% of dysgerminomas. In all, 57% of cases had only isochromosome 12p and 5% had only 12p overrepresentation. In all, 19% had both isochrome 12p and 12p overrepresentation. Gonadoblastomas were negative for isochromosome 12p or 12p overrepresentation. Chromosome 12p abnormalities are common in dysgerminoma of the ovary. FISH analyses for chromosome 12p abnormalities may be a useful diagnostic adjunct for confirming the diagnosis of dysgerminoma and for distinguishing it from nongerm cell malignancies that enter into the differential diagnosis.
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Ovarian germ cell tumors comprise a heterogeneous group of benign and malignant neoplasms, among which dysgerminoma is the most common malignant tumor. Dysgerminoma represents the ovarian counterpart of testicular seminoma.[1] Unlike seminoma, dysgerminoma has not been well characterized genetically due to the scarcity of cases and the difficulty in obtaining fresh material necessary to perform classical cytogenetic analyses.

Gain of genetic material from the short arm of chromosome 12 is an early molecular event in the evolution of testicular germ cell tumors, and there is additional evidence that 12p overrepresentation is involved in the development of invasive potential.[2–5] The hallmark genetic marker of testicular germ cell tumor is the presence of an isochromosome of the short arm of chromosome 12 (i(12p)), first recognized using karyotypic analysis by Atkin and Baker[6,7] who described a small metacentric marker in a small series of seminomas and subsequently in nonseminomatous germ cell tumors. Very little is known about the genetic abnormalities of dysgerminoma. In this study, we analyze chromosome 12p abnormalities in a series of 21 ovarian dysgerminomas and two gonadoblastomas with dual-color fluorescence *in situ* hybridization (FISH) techniques on formalin-fixed, paraffin-embedded specimens.

Materials and methods

Specimens

A total of 21 cases of ovarian dysgerminomas were retrieved from the files of the Departments of Pathology of three different Institutions (Indiana University Medical Center, Indianapolis, IN, USA; University Hospitals of Cleveland, Clevleland, OH, USA; and Yale University, New Haven, CT, USA). The patients' ages ranged from 10 and 50 years (mean, 23 years). The FIGO staging system for

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ovarian neoplasms was used.[8] Pathological stage was recorded as 1a in 14 cases, 1c in one case, 2a in one case and 3c in the remaining five cases, among which four had lymph node metastases and one showed peritoneal disease. Histologically, all of the tumors were pure dysgerminomas. Additionally, two cases of gonadoblastoma from a 13-year-old and a 15-year-old patients were evaluated in this study.

Tissue Preparation and Fluorescence *In Situ* Hybridization

Fluorescence *in situ* hybridization (FISH) was performed as previously described.[9–12] Sections (4 μm) were prepared from buffered, formalin-fixed, paraffin-embedded tissue blocks. The slides were deparaffinized with two washes of xylene, 15 mins for each, and the slides were subsequently washed twice with absolute ethanol, 10 min each. The slides then were air dried in the hood. Next the slides were treated in 0.1 mM citric acid (pH6.0) (Zymed, CA, USA) at 95°C for 10 min, rinsed in distilled water for 3 min, and followed by a wash of 2 × SSC (standard saline citrate) for 5 min. Digestion of the tissue was performed by applying 0.4 ml of pepsin (5 mg/ml in 0.9% NaCl, pH 1.5) (Sigma, St Louis, MO, USA) at 37°C for 40 min. The slides were rinsed with distilled water for 3 min and further washed with 2 × SSC for 5 min, and then allowed to air dry.

Dual-color FISH was performed by using a mixture of a Spectrum orange-labeled Centromeric α-satellite DNA probe (CEP12) and a Spectrum green-labeled subtelomeric (Tel12) DNA probe for chromosome 12p. Both of the probes were from Vysis (Vysis, Downers Grove, IL, USA) and were diluted with tDenHyb2 (Insitus, Alburquerque, NM, USA) in a ratio of 1:50 and 1:20, respectively. Diluted probes (5 μl) were added to the slide in the reduced light condition. The slides were covered with a 22 × 22 mm cover slip and sealed with rubber cement. Denaturation was achieved by incubating the slides at 75°C for 10 min in a humidified box and then hybridized at 37°C over night.

The cover slips were removed and the slides were washed extensively twice with 45°C prewarmed 0.1 × SSC/1.5 M urea, 20 min for each. This was followed by a wash with 2 × SSC for 20 min and with 2 × SSC/0.1% NP40 for 10 min at 45°C. The slides were then further washed with room temperature 2 × SSC for 5 min. The slides were air dried and counterstained with 10 μl DAPI (Insitus, Albuquerque, NM, USA). The slides were covered and sealed with nail polish.

The slides were examined using a Zeiss Axioplan 2 microscope (Ziess, Göttingen, Germany) with the following filters: SP-100 DAPI, FITC MF-101 for spectrum green (12p) and Gold 31003 for Spectrum orange (CEP12) from Chroma (Chroma, Brattleboro, VT, USA).

The images were acquired with a CCD camera and analyzed with MetaSystem Isis software (MetaSystem, Belmont, MA, USA). Five sequential focus stacks with 0.4 μm interval were acquired and then integrated into a single image in order to reduce thickness related artifacts.

From each tumor section, 100 nuclei were scored for signal from CEP12 (red) and 12p (green) under the fluorescence microscope with × 1000 magnification and the ratio between green and red signals was subsequently calculated. The quantitative criteria to determine 12p overrepresentation were previously described.[12]

We analyzed the spatial distribution of the green and red signals to detect the specific patterns of signal aggregation consistent with i(12p), as previously reported.[13–16] A classical seminoma specimen was used as a positive control for FISH analyses, and lymphocytes in dysgerminoma specimens were used for the negative control. The positive control specimen represented by a classical seminoma showed both overrepresentation of 12p and the presence of i(12p) in a small percentage of nuclei, while lymphocytes from the background of dysgerminomas were consistently negative for 12p overrepresentation and for i(12p).

Statistical Analysis

Data were analyzed with SAS software (SAS Institute Inc., Cary, NC, USA). The presence of 12p abnormalities were correlated with other clinicopathologic variables. Since some variable is discrete and not continuous, a multivariate logistic regression model was constructed using a backward stepwise selection procedure. The variables were first analyzed by χ^2 method and the odd ratio was calculated. The variables were then included into a complete model, and were progressively eliminated in order to obtain the parsimonious model with the best overall predictive power. A P-value <0.05 was considered statistically significant.

Results

All of the slides showed well-defined hybridization signals. Chromosome 12p abnormalities were detected in 17 of 21 (81%) of dysgerminomas (Figure 1). We observed overrepresentation of 12p in 5 of 21 dysgerminomas (24%), while in 16 of 21 cases (76%) demonstrated i(12p) in a variable percentage of nuclei ranging from 2 to 5%. Among the i(12p)-positive cases, Four cases (25%) also had 12p overrepresentation. Four of five cases (80%) with 12p overrepresentation also had i(12p). Only four of 21 (19%) cases showed neither 12p overrepresentation nor the presence of i(12p). Morphologically, we could not identify difference between dysgerminomas with chromosome 12p abnormalities and dysgerminomas without i(12p) or 12p overrepresentation.

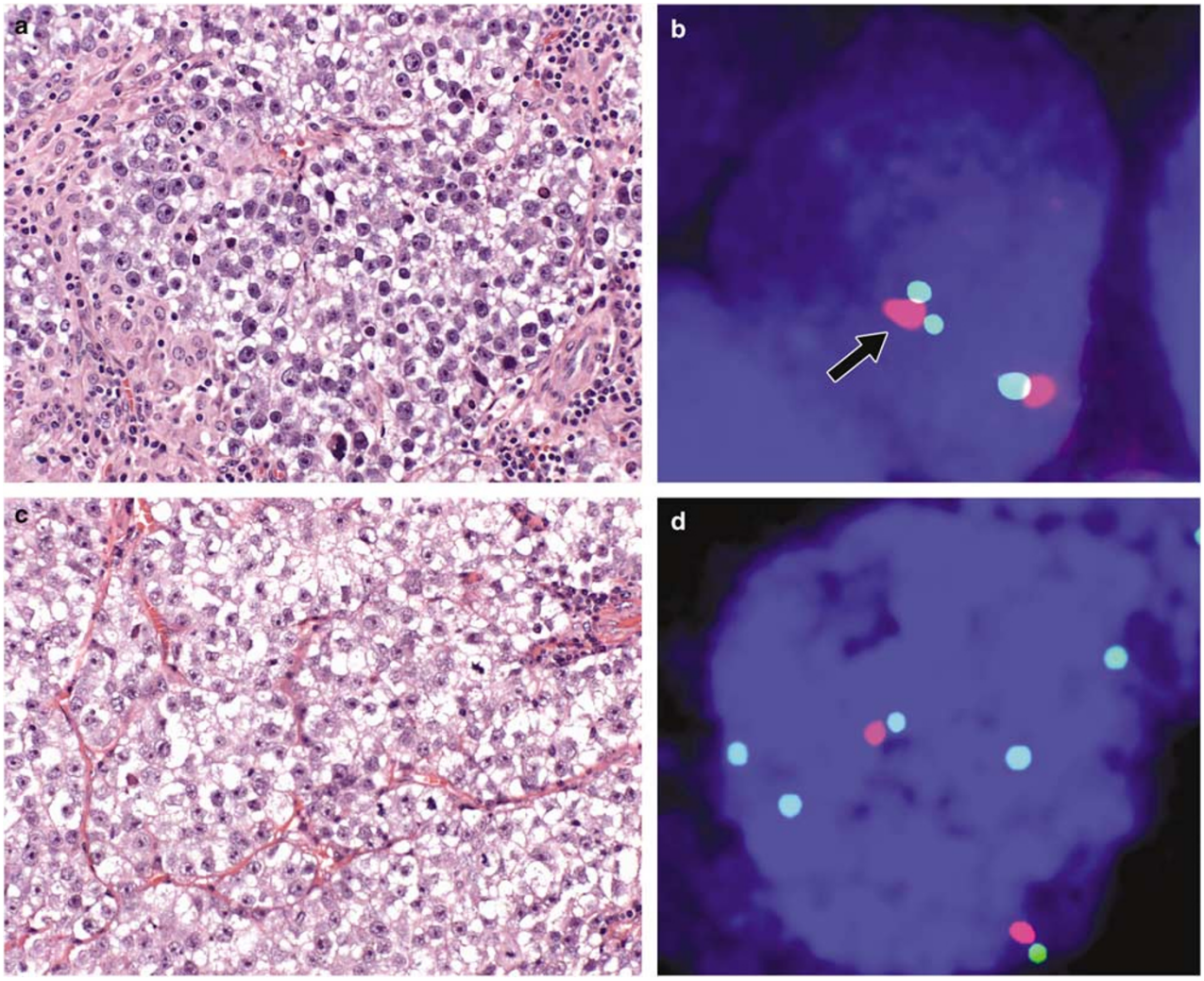

Figure 1 (**a**) and (**c**) Classic dysgerminoma showing typical dysgerminoma cells with well defined cell borders, arranged in nests delimited by fibrovascular septa containing a variable number of small lymphocytes. (**b**) Fluorescence *in situ* hybridization displayed two signals (orange) for the chromosome 12 centromeric probe and three signals (green) for 12p, two of which were in close proximity to one centromeric signal, with an aggregation pattern (arrow) consistent with an isochromosome 12p. (**d**) Fluorescence *in situ* hybridization showed two signals (orange) for the chromosome 12 centromeric probe and numerous signals for 12p (green) subtelomeric probe, as observed in 12p overrepresentation.

FISH results and pathological stages are summarized in Table 1. No correlation was found between 12p abnormalities and various clinicopathologic parameters including pathologic stage (all *P*-value >0.05).

Neither 12p overrepresentation nor the presence of i(12p) was observed in two gonadoblastomas.

Discussion

The hallmark genetic markers of testicular germ cell tumors are chromosome 12p anomalies, including isochromosome 12p and 12p overrepresentation. However, similar genetic markers for ovarian germ cell tumors are less well-characterized. We analyzed a large series of ovarian dysgerminomas by dual color FISH technologies and found 12p abnormalities in 81% of cases. Additionally, we also analyzed two gonadoblatoma for 12p abnormalities using FISH and both were negative for these genetic abnormalities. Our findings suggest that dysgerminoma shows the same genetic markers as its testicular counterpart, the seminoma, and probably shares the same pathogenetic pathways.

There are relatively few reports of genetic abnormalities in dysgerminoma. Jenkyn and McCartney[17] reported the results of karyotypic analyses on three different types of malignant ovarian tumors, and i(12p) was detected on ascitic fluid cells from a 19-year-old girl with dysgerminoma. At the same time, Atkin and Baker demonstrated the presence of i(12p) as a peculiar genetic abnormality in one dysgerminoma.[18] Dal Cin *et al* reported a dysgerminoma with overrepresentation of 12p as a small metacentric derivative of chromosome 12.[19] Abnormalities

Table 1 FISH results for Chromosome 12p analysis

Cases	*i(12p)*	*12p OR**	*Stage*
1	Pos	Neg	1a
2	Pos	Neg	1a
3	Pos	Neg	1a
4	Pos	Pos	3c
5	Neg	Neg	1a
6	Pos	Neg	1c
7	Pos	Pos	3c
8	Neg	Neg	1a
9	Pos	Neg	1a
10	Pos	Neg	2a
11	Neg	Pos	1a
12	Pos	Neg	1a
13	Neg	Neg	3c
14	Pos	Pos	1a
15	Pos	Pos	1a
16	Pos	Neg	3c
17	Pos	Neg	1a
18	Pos	Neg	1a
19	Pos	Neg	1a
20	Neg	Neg	3c
21	Pos	Neg	1a
22[§]	Neg	Neg	1b
23[§]	Neg	Neg	1b

*: chromosome 12 overrepresentation. No correlation was found between 12 abnormalities and pathologic stage.
[§]Gonadoblastoma.

of 12p in dysgerminoma were further described by Rodriguez *et al*[20] in a series of three ovarian germ cell tumors and by Riopel *et al*,[21] who reported a gain of 12p in two dysgerminomas. More recently, Kraggerud *et al*[22] confirmed that a gain of 12p was the most common change in dysgerminoma using the comparative genomic hybridization (CGH) approach.

Previous studies have demonstrated that FISH analysis might be helpful in defining 12p overrepresentation as a diagnostic tool in the differential diagnosis of metastatic germ cell tumors.[12] An accurate diagnosis of dysgerminoma is important for clinical management as these tumors have a better prognosis than other primary ovarian malignancies, are radiosensitive, and have a good response to cisplatin-based chemotherapy regimens. The differential diagnosis of dysgerminoma includes other histologic types of ovarian germ cell tumors, mainly solid variants of yolk sac tumor and embryonal carcinoma, but also non-germ cell tumors, such as granulosa cell tumor, undifferentiated carcinoma, clear cell adenocarcinoma, and lymphoma, as well as metastatic tumors such as melanoma and breast carcinoma.[23] Molecular genetic studies on granulosa cell tumors have frequently identified chromosomal abnormalities as monosomy for chromosome 22 and trisomy for chromosomes 14 and 12,[24,25] but neither i(12p) nor 12p overrepresentation has been reported, whereas extensive genetic characterizations of undifferentiated carcinoma and clear cell adenocarcinomas are still lacking. Positive immunostainings for OCT4 is useful for supporting the diagnosis of dysgerminoma;[26] however, a significant proportion of clear cell adenocarcinomas of the ovary are also positive for OCT4.[23] We demonstrated that 12p anomalies are detected in the majority of dysgerminomas. Therefore, FISH analysis for 12p anomalies may be a useful diagnostic adjunct in cases where dysgerminoma is considered in the differential diagnosis.

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