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Short Communication

Trisomy 12 in Pediatric Granulosa-Stromal Cell Tumors

Demonstration by a Modified Method of Fluorescence In Situ *Hybridization on Paraffin-embedded Material*

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The use of fluorescence in situ hybridization (FISH) to detect chromosomal abnormalities has many applications. Use of FISH on archival, paraffin-embedded material has been limited to microscopic sections. We have carried out FISH on preparations of disaggregated nuclei obtained from paraffin-embedded tissue to evaluate chromosome 12 copy number in granulosa-stromal cell neoplasms occurring in infants, children, and adolescents. Trisomy 12 was detected in the majority of cells from three of four juvenile granulosa cell tumors (three ovarian and one testicular) and one malignant granulosa cell tumor. Tetrasomy 12 was observed in a case of ovarian thecoma. (Am J Pathol 1992, 141:1265–1269)

Fluorescence *in situ* hybridization (FISH) has become a powerful tool in both diagnostic and experimental molecular cytogenetics. In clinical medicine, detection of specific RNA and DNA sequences within interphase and metaphase cells has been useful in many areas, including detection of viral infections[1] and prenatal diagnosis of chromosomal abnormalities.[2] Demonstration of aneuploidy and chromosomal translocations by FISH has clinical relevance in the field of tumor diagnosis.[3,4]

Utilization of microscopic sections of paraffin-embedded material for *in situ* hybridization allows histologic localization of the signal to a specific cell or nucleus. Although this technique can be done in archival material, it has two major drawbacks. One is that variations in tissue type, processing, and handling all influence the effectiveness of hybridization. The other drawback is that only portions of individual cells (or nuclei), rather than intact cells (or nuclei) are examined in a given 3–5 μm thick section, making quantitative studies difficult. The latter prompted us to use disaggregated, paraffin-embedded tissue for our analysis.

Juvenile granulosa cell tumors (JGCT) of the ovary and testis are uncommon neoplasms. These tumors frequently are grossly cystic and characterized histologically by the presence of variably sized follicles containing mucin. The neoplastic cells have round, hyperchromatic nuclei that lack conspicuous grooves.[5] Reports[6,7] indicate that trisomy 12 may be a nonrandom aberration in ovarian granulosa cell tumors (GCTs). Karyotypic data have not been reported for testicular GCTs.

Materials and Methods

Four cases of unilateral juvenile granulosa cell tumor of the ovary were identified in girls aged 18 months to 11 years. There was no known history of an associated/predisposing condition (such as Maffucci's or Ollier's dis-

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ease) in any of the children. A malignant GCT of the ovary with intraabdominal metastases, a case of JGCT originating in an intraabdominal testes of a male infant and a case of ovarian thecoma occurring in an adolescent female with Down's syndrome completed the study group. All specimens were obtained at surgery other than the malignant GCT, which was diagnosed at autopsy. The postmortem interval in this case was 3 hours. The cases are summarized in Table 1.

The histology of all JGCTs (ovarian testicular) was typical for this entity in that there were numerous mucin-containing follicles of various sizes and shapes (Figure 1). Unlike the GCT found in adult women, nuclear grooves were not conspicuous. Follicles were small and few in the MGCT, which was characterized by solid sheets, nests, and trabeculae of small cells with multiple foci of necrosis. The nuclei were oval to angular in shape and mitoses were frequent. The ovarian thecoma was comprised of spindle-shaped cells with abundant pale cytoplasm arranged in ill-defined bundles and fasicles.

Routine karyotypic analysis was available for one of the ovarian JGCT (case 3) and has been previously published (case 8).[7]

After review of H&E-stained slides prepared from the tumors, a representative block was selected for analysis. Depending on the amount of tumor tissue present, one to three 60-μm sections were cut and disaggregated according to the method of Hedley et al.[8] Briefly, deparaffinization in xylene was followed by rehydration to distilled water in graded alcohols (100, 90, 70, and 50%). The sections were then digested with pepsin (Sigma, St. Louis, MO, catalog #P-6887) for 30–60 minutes in a 37° C water bath. The tissue was then vigorously drawn up and down in a 5-mL syringe with an 18-gauge needle and filtered through nylon mesh (Small Parts Inc., Miami, FL—#CMN-105). The samples were rinsed with 4 mL of phosphate-buffered saline (PBS) and centrifuged at 1000 rpm. Resuspension in 2 mL of PBS was followed by "syringing" with a 21-gauge needle and recentrifugation at 1000 rpm for 10 minutes. The resulting nuclear pellet was resuspended in a small amount of PBS and pipetted onto a glass slide. The slides were air-dried 1 to 14 days at room temperature before hybridization.

The remainder of the reagents were obtained from Oncor, Gaithersburg, MD. The hybridization, signal detection, and amplification were carried out according to the manufacturer's published protocol. After rinsing for 2 minutes in 2X SSC, the slides were dehydrated by sequential 2-minute rinses in graded alcohols (70, 80, 90, and 100%). A final 2-minute rinse in acetone was done before allowing the slides to air dry for approximately 1 hour. The slides were then incubated overnight in a 37°C incubator with a mixture of biotinylated probe (chromosome 12 alpha satellite—*D12Z3;* Y chromosome classic satellite—*DYZ1*) and Hybrisol VI at a concentration of 30 ng probe/μl. After detection and amplification (Oncor, Gaithersburg, MD, cat #S1352-SET), the results were visualized with a Zeiss Axiophot fluorescent microscope (Carl Zeiss, Oberkochen, FRG) using a BP 450-490 excitation filter, a FT 510 beam splitter, and a LP520 barrier filter.

Results

One case of ovarian JGCT that was biopsied and resected in 1968 and preserved in Zenker's fixative (6a and 6b) yielded few cells after disaggregation and the residual nuclei did not hybridize with the chromosome 12 centromeric probe (Table 2).

The remaining six cases, processed between 1966 and 1991, were characterized by well-preserved nuclei that yielded bright, easily interpretable hybridization signals (Figure 2). Trisomy 12 was observed in two of three ovarian JGCTs and the MGCT (both the ovarian primary and a liver metastasis). As previously noted, karyotypic analysis corroborated the presence of three copies of chromosome 12 in case 3 (47, XX, +12). Tetrasomy 12 characterized the single case of ovarian thecoma. Disaggregated nuclei from the testicular granulosa cell tumor had three signals in approximately 50% of nuclei when hybridized with *D12Z3,* but uniformly had one signal when hybridized with the Y chromosome probe.

Discussion

Ovarian granulosa cell tumors (GCT) account for approximately 2% of all ovarian neoplasms. Those occurring in

Table 1. *Clinical data*

	Age/Sex	Diagnosis/Site	Year of diagnosis	Tissue fixative
Case 1	1 mo/M	JGCT/T	1991	10% Formalin
Case 2	5 yr/F	JGCT/O	1991	10% Formalin
Case 3	7 yr/F	JGCT/O	1990	10% Formalin
Case 4	20 yr/F	Thecoma/O	1980	10% Formalin
Case 5*	7 yr/F	MGCT/O	1968	10% Formalin
Case 6	11 yr/F	JGCT/O	1968	Zenker's
Case 7	18 mo/F	JGCT/O	1966	10% Formalin

* Autopsy; others are surgical specimens.
M = male; F = female. JGCT = juvenile granulosa cell tumor, MGCT = malignant granulosa cell tumor, O = ovary; T = testis.

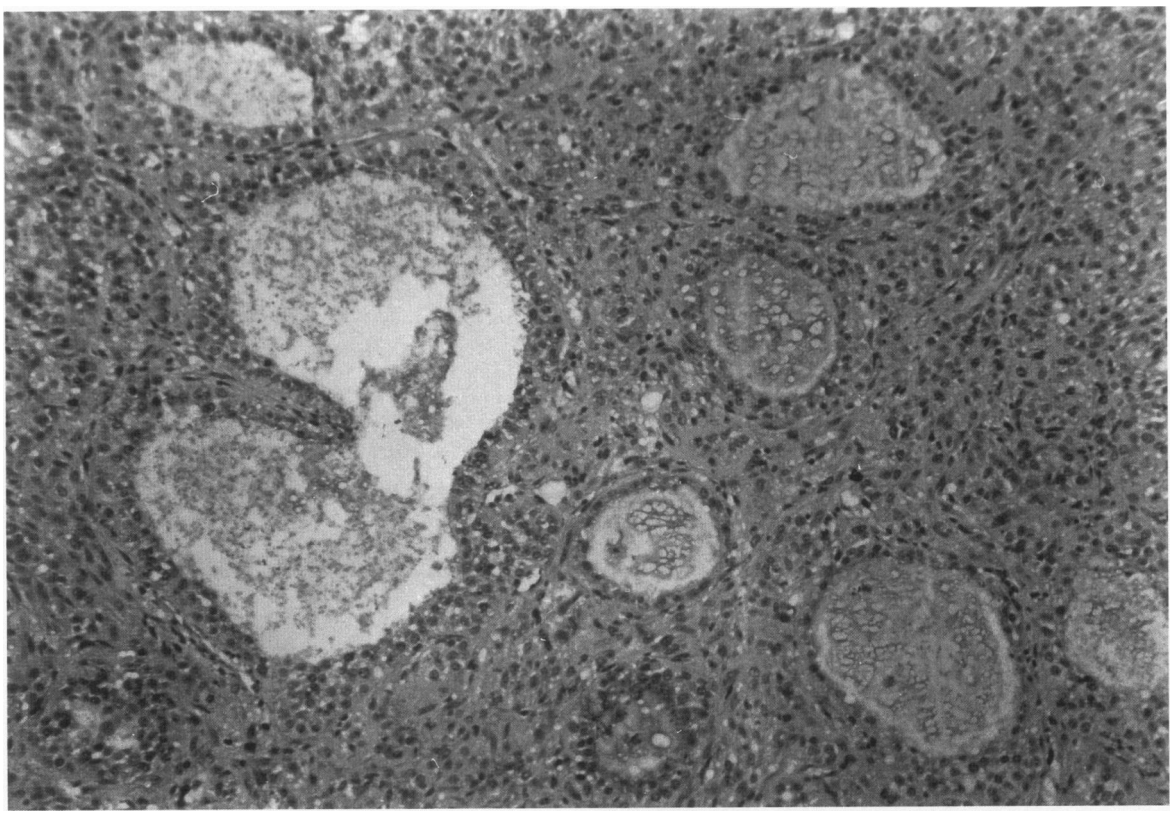

Figure 1. *Grossly, the JGCTs had both solid and cystic regions. The cystic component predominated in most cases and, as illustrated here (case 2), was histologically characterized by numerous variably sized follicles containing mucin (H&E stain, ×100).*

children and adolescents frequently have a more cystic appearance than their adult counterparts and have been designated juvenile GCTs. Histologically, JGCTs are characterized by numerous follicles, frequent leuteinization, and cells with round nuclei that lack prominent grooves. Although associated with a benign clinical course, occasional malignant GCTs are encountered.[5,9]

Trisomy 12 has been reported as a feature of ovarian fibrothecomas and granulosa cell neoplasms, suggesting that this may be a nonrandom cytogenetic aberration.[6,7,10,11] This abnormality is not universal, as bilateral fibrothecomas occurring in a 65-year-old female were karyotypically normal.[7]

We used FISH with a probe for the *D12Z3* alpha satellite region of chromosome 12 to assess chromosome 12 copy number in granulosa-stromal cell neoplasms occurring in infants, children, and adolescents. Three primary nuclear hybridization signals were observed in three of four cases of JGCT and a single case of MGCT. Trisomy for chromosome 12 was similarly documented in

Table 2. *Hybridization Results*

	Number of signals/nucleus						
	0	1	2	3	4	>4	Total
Case 1	5	4	86	103	2		200
Case 1*	9	189	1				200
Case 2	8	1	186	3	2		200
Case 3	2	1	46	147	4		200
Case 4	2	6	37	30	122	3	200
Case 5a	16	1	41	130	12		200
Case 5b	45	5	58	80	12		200
Case 6a	35						35
Case 6b	40						40
Case 7	6	4	68	119	3		200

DYZ1 (Chromosome Y) probe (rest are D12Z3 (chromosome 12) probe).
Case 5a = ovarian primary; 5b = liver metastasis.
Case 6a = ovarian biopsy; 6b = subsequent oophorectomy.

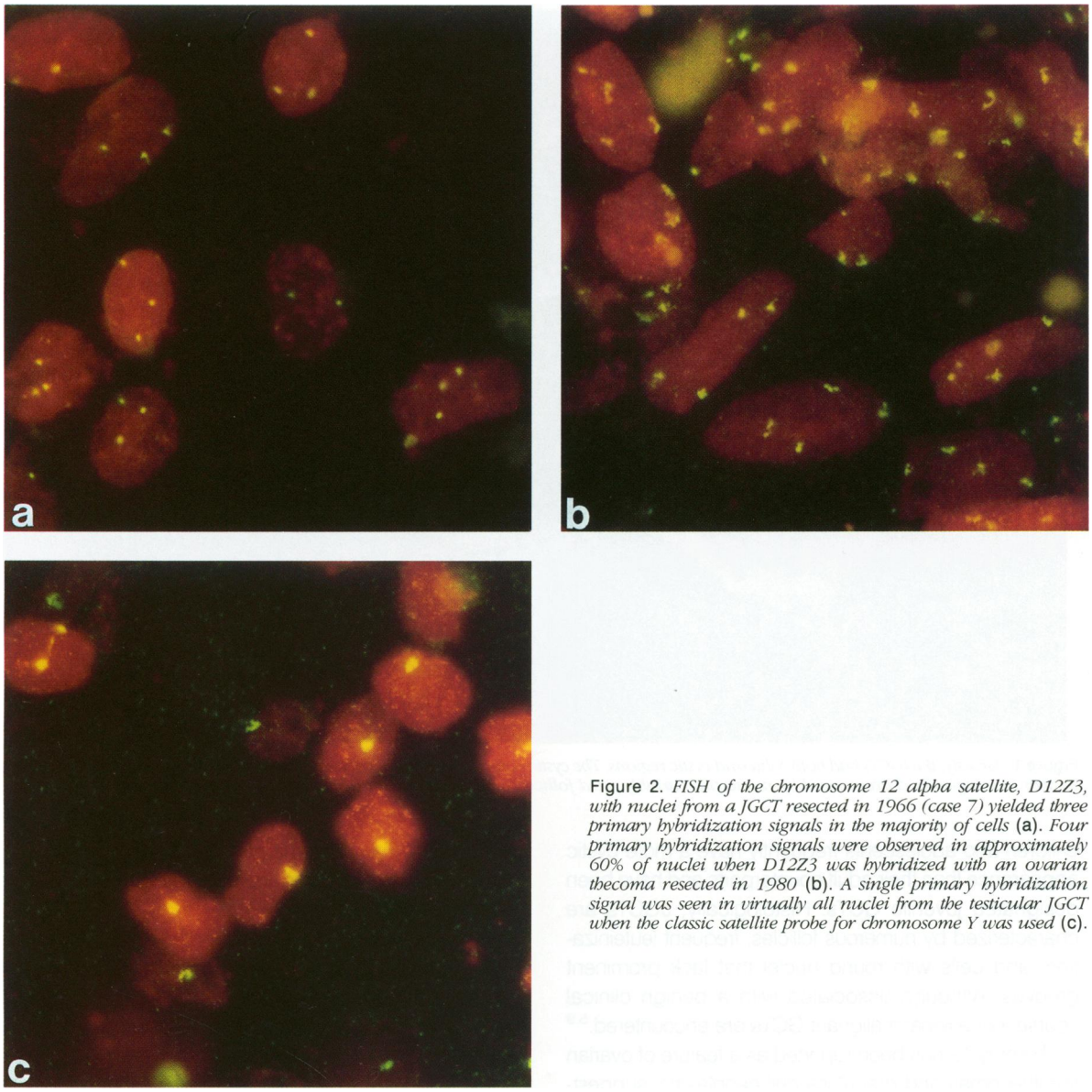

Figure 2. *FISH of the chromosome 12 alpha satellite, D12Z3, with nuclei from a JGCT resected in 1966 (case 7) yielded three primary hybridization signals in the majority of cells* (a). *Four primary hybridization signals were observed in approximately 60% of nuclei when D12Z3 was hybridized with an ovarian thecoma resected in 1980* (b). *A single primary hybridization signal was seen in virtually all nuclei from the testicular JGCT when the classic satellite probe for chromosome Y was used* (c).

a liver metastasis in the MGCT. One case of ovarian JGCT (with classic histology) had only two hybridization signals, suggesting one of two possibilities. Either a minority of these neoplasms lack duplication of chromosome 12 material, or duplicated material from chromosome 12, which did not include the *D12Z3* alpha satellite region, was present.

Tetrasomy for chromosome 12 was suggested by the four primary hybridization signals observed in the case of ovarian thecoma. This is not surprising as karyotypic data reported previously includes 1 of 14 fibrothecomas with 4 copies of chromosome 12.[6,7,10,11]

Documentation of trisomy 12 in a JGCT of the testis in an infant suggest that, despite their clinical dissimilarities, some of these neoplasms might share more than a common histologic appearance with their ovarian counterparts.[12,13] Due to reports that a subset of these tumors occur in males with gonadal dysgenesis[14] and a constitutional 46XY/45XO mosaicism, we investigated the possibility that loss of a Y chromosome was a feature of this tumor. Despite origin in an undescended abdominal testis, the infant did not have clinical features to suggest gonadal dysgenesis and a single Y chromosome signal was observed in virtually all nuclei examined. Although consistent with the presence of a normal Y chromosome, this finding would not exclude the possibility that loss of a portion of this sex chromosome is contributory to the pathogenesis of these neoplasms.

In addition to establishing the nonrandom occurrence of trisomy 12 in JGCTs, we have illustrated the potential

usefulness of FISH on disaggregated, paraffin-embedded material. Although signal intensity varied from case to case, they were easily interpreted and counted, including tissue obtained at autopsy and tissue processed at least 25 years previously. Correlation of the percentage of tumor cells on the H&E-stained section with the percentage of cells having an abnormal number of signals aids in interpretation of the results. Our FISH analyses were unsuccessful using tissue preserved in Zenker's fixative. However, storage conditions (variations in temperature, moisture, etc.) of these blocks were unknown. Analysis of additional specimens will demonstrate whether certain fixatives interfere with the FISH approach described herein.

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