Interphase Fluorescence \textit{In situ} Hybridization Analysis of Chromosome 12p Abnormalities Is Useful for Distinguishing Epidermoid Cysts of the Testis from Pure Mature Teratoma

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Abstract

\textbf{Purpose:} The distinction of epidermoid cyst of the testis from teratoma is of critical importance because the former is benign and the latter is a malignant tumor that may have associated metastasis of either teratomatous or non–teratomatous germ cell tumor types. Chromosome 12p abnormalities are seen in the vast majority of testicular germ cell tumors of adults and are present in all histologic subtypes. In this study, we investigated the clinical utility of interphase fluorescence \textit{in situ} hybridization (FISH) analysis of chromosome 12p abnormalities for distinguishing epidermoid cysts of the testis from pure mature teratoma.

\textbf{Experimental Design:} Sixteen testicular epidermoid cysts and 17 testicular teratomas were investigated for isochromosome 12p \([i(12p)]\) and 12p overrepresentation using interphase FISH analysis.

\textbf{Results:} Neither \([i(12p)]\) nor 12p overrepresentation were observed in 16 epidermoid cyst cases, whereas \([i(12p)]\) was detected in 76\% of teratomas and 12p overrepresentation was identified in 29\% of teratomas. Overall, 88\% of testicular teratomas had chromosome 12p abnormalities.

\textbf{Conclusions:} FISH identification of \([i(12p)]\) and/or 12p overrepresentation in routinely processed surgical specimens is a useful ancillary diagnostic tool in distinguishing testicular epidermoid cysts from teratoma.

Epidermoid cyst of the testis is an uncommon benign lesion accounting for $<$1\% of all testicular tumors (1). Several authors have hypothesized that it originates from squamous metaplasia of the mesothelium, rete testis epithelium, or seminiferous tubular cells; others believe that it represents a monodermal teratoma (1, 2). The main differential diagnostic consideration of epidermoid cyst is teratoma of the testis. This distinction is critically important for prognosis and patient management. Unlike its ovarian counterpart, teratoma of the testis is a malignant neoplasm with a high incidence of metastasis. After complete removal of the tumor by orchietomy, additional treatments, including retroperitoneal lymph node dissection and chemotherapy, may be considered for patients with testicular teratoma. In contrast, complete removal of the lesion by surgery is curative for epidermoid cyst of the testis (3).

Distinguishing testicular epidermoid cyst from teratoma can be a challenging exercise. A number of well-established histologic criteria have been proposed for use in making this distinction (4). Other than these proposed histologic criteria, no useful diagnostic techniques have been developed to aid in this diagnostic dilemma. It would be helpful to identify a sensitive and specific molecular test, applicable to paraffin-embedded tissue, which would assist in this process.

Chromosome 12p abnormalities (either as isochromosome 12p \([i(12p)]\) or 12p overrepresentation) are seen in the vast majority of testicular germ cell tumors and have been observed in all histologic subtypes (5, 6). In this study, we investigated whether interphase fluorescence \textit{in situ} hybridization (FISH) analysis of chromosome 12p abnormalities is useful in distinguishing epidermoid cysts of the testis from testicular teratoma using paraffin-embedded tissue.

Materials and Methods

\textbf{Patients and specimens.} Sixteen cases of prepubertal and postpubertal testicular epidermoid cyst and 17 cases of postpubertal pure testicular teratoma were retrieved from the surgical pathology files of the Indiana University School of Medicine (Indianapolis, IN) and Case Western Reserve University (Cleveland, OH) from 1992 to 2000. One epidermoid cyst in a seven-year-old boy was treated by partial
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FISH analysis. FISH test was done as previously described (7–11). Four-micrometer sections were prepared from buffered formalin-fixed, paraffin-embedded tissue blocks. The slides were deparaffinized with two 15-minute washes of xylene, and were subsequently washed twice with absolute ethanol for 10 minutes each. The slides then were air-dried in a fume hood. Next, the slides were treated in 0.1 mmol/L of citric acid (pH 6.0; Zymed, Carlsbad, CA) at 95°C for 10 minutes, rinsed and distilled water for 3 minutes, and washed with 2× SSC for 5 minutes. Digestion of the tissue was done by applying 0.4 mL of pepsin (5 mg/mL in 0.9% NaCl, pH 1.5; Sigma, St. Louis, MO) at 37°C for 40 minutes. The slides were rinsed with distilled water for 3 minutes, washed again with 2× SSC for 5 minutes, and air-dried.

Dual-color FISH was done by using a mixture of Spectrum Orange–labeled centromeric α satellite DNA probe (CEP12) and Spectrum Green–labeled subtelomeric (Tel12) DNA probes for chromosome 12p. Both of the probes were from Vysis (Downers Grove, IL) and were diluted with dTenHyb2 (Insius, Albuquerque, NM) in a ratio of 1:50 and 1:20, respectively. Five microliters of diluted probes were added to the slide in the reduced light condition, slides were covered with a 22 × 22 mm coverslip, and were sealed with rubber cement. Denaturation was achieved by incubating the slides at 75°C for 10 minutes in a humidified box, then the slides were hybridized at 37°C overnight.

The coverslips were removed and the slides were washed extensively twice with 45°C prewarmed 0.1× SSC/1.5 mol/L urea, for 20 minutes for each, followed by a wash with 2× SSC for 20 minutes and 2× SSC/0.1% NP40 for 10 minutes at 45°C. The slides were further washed with room temperature 2× SSC for 5 minutes. The slides were air-dried and counterstained with 10 μL of 4’,6-diamidino-2-phenylindole (DAPI–Antifade Insitus). The slides were covered and sealed with nail polish.

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

The images were acquired with a CCD camera and analyzed with MetaSystem Isis software (Belmont, MA). Five sequential focus stacks with 0.4-μm intervals 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 signals from CEP12 (red) and 12p (green) under the fluorescence microscope with the following filters: SP-100 DAPI, FITC Orange (CEP12) from Chroma (Brattleboro, VT). The quantitative criteria to determine 12p overrepresentation have been described previously (15–17).

A classical seminoma specimen was used as a positive control for FISH analyses. Lymphocyte and stromal nuclei from the same tumor were used as normal controls for each tumor. In addition, we analyzed six cases of skin punch biopsies from patients without a history of germ cell tumors.

Results

Sixteen cases of prepubertal and postpubertal testicular epidermoid cyst and 17 cases of postpubertal testicular teratoma were investigated for i(12p) and 12p overrepresentation. Patients with epidermoid cyst ranged in age from 7 to 44 years old (mean, 22) and those with mature teratoma ranged in age from 23 to 46 years old (mean, 33) at the time of the initial diagnosis.

All of the slides showed well-defined hybridization signals. Neither i(12p) nor 12p overrepresentation was observed in 16 epidermoid cyst cases (Fig. 1). Overall, 88% of testicular teratomas had chromosome 12p abnormalities (either i(12p) and/or 12p overrepresentation; Table 1). i(12p) was detected in 13 of 17 (76%) teratomas in a percentage of nuclei ranging from 2% to 5%, and 12p overrepresentation was observed in 5 of 17 mature teratomas (29%; Fig. 1). Among the 13 cases of i(12p)-positive mature teratomas, 3 cases (24%) also had 12p overrepresentation. Three of the five cases (60%) with 12p overrepresentation also had i(12p). Only 2 of 17 cases (12%) showed neither 12p overrepresentation nor the presence of i(12p) (Table 1). Neither i(12p) nor 12p overrepresentation was observed in lymphocytes or stromal cells from the study patients or keratinocytes from skin punch biopsy specimens.

Discussion

Epidermoid cysts were first described in 1942 by Dockerty and Priestly (18). They are uncommon, and are composed of cells lined by squamous epithelium. Epidermoid cysts account for ~1% of adult testicular tumors (1) and 3% of pediatric testicular tumors (3), although in some studies, they have comprised up to 4% of testicular tumors (19). Many authors consider them to be monodermal teratomas (1, 4). Evidence supporting the teratomatous nature of testicular epidermoid cysts includes the fact that the age range of their occurrence overlaps with that of recognized germ cell tumors, and that morphologically typical epidermoid cysts are sometimes found to be components of classic teratomas. However, unlike the majority of teratomas in males, epidermoid cysts are not associated with mitotic activity, cytologic atypia, intratubular germ cell neoplasia of the unclassified type, or testicular atrophy (4), and uniformly exhibit benign clinical behavior (1, 2, 20–22). Price (1) reported a large series of 69 cases of testicular epidermoid cysts, all of which were characterized by an unremarkable clinical course, with a period of follow-up ranging from 1 to 24 months (22). Because epidermoid cyst of the testis is a benign tumor that is amenable to conservative therapy, as opposed to testicular teratoma, which requires radical orchietomy, evaluation for metastasis, and sometimes adjuvant chemotherapy, it is of paramount importance to distinguish one from the other.

The hallmark genetic markers of testicular germ cell tumors are chromosome 12p abnormalities, including i(12p) and 12p overrepresentation (6). Abnormalities of 12p have been shown to occur early in the evolution of germ cell tumor (23, 24). Recent studies have shown that FISH analysis is the most sensitive method to detect i(12p) (24). In this study, we analyzed a series of 16 epidermoid cysts and 17 testicular teratomas by dual-color FISH technologies and found 12p abnormalities in 88% of teratoma cases but none in the 16 epidermoid cyst cases. Our study showed that testicular epidermoid cysts are genetically different from testicular teratomas and interphase FISH analysis of chromosome 12p abnormalities may be useful to distinguish testicular epidermoid cysts from testicular teratomas. When a testicular tumor is managed by radical orchietomy, the distinction between teratoma and epidermoid cyst is not usually difficult and probably does not require FISH analysis; however, conservative surgery may be indicated in patients with cystic masses, solitary testis, or infertility, in an effort to preserve testicular parenchyma. In these cases, the pathologist may have a more difficult time distinguishing between the two entities, and the use of...
FISH analysis could aid in the distinction of these entities in locally excised masses. However, teratomas of the testis in prepubertal boys are not malignant, and thus, the distinction between teratoma and epidermoid cyst is not critical in this age group.

The genetic abnormalities associated with epidermoid cysts have not been extensively investigated. A study by Younger et al. showed that epidermoid cysts have different loss of heterozygosity patterns from malignant germ cell tumors, emphasizing a lack of similarity with these tumors (25). In contrast, the genetic abnormalities associated with testicular germ cell tumors have been thoroughly evaluated, including many studies that have evaluated 12p abnormalities in testicular germ cell tumors. These studies include classic cytogenetic analyses, FISH on metaphase cells of fresh tissue, and comparative genomic hybridization. Standard cytogenetics detects i(12p) in 50% to 70% of testicular germ cell tumors (26). The i(12p)-negative cases, however, showed other 12p abnormalities (27). Using FISH, Smolarek et al. (28), evaluated 11 primary germ cell tumors and 16 metastatic germ cell tumors involving lymph nodes, and found overrepresentation of 12p in all 27 tumors. Other studies, including comparative genomic hybridization, have also characterized the overrepresentation of 12p in testicular germ cell tumors. Mostert et al. examined 15 primary testicular germ cell tumors and found that 12p abnormalities in the majority of the tumors involved the complete short arm of chromosome 12 (29). Abnormalities of 12p in testicular teratomas have been shown to occur early in the evolution of

![Fig. 1.](image)

A, testicular teratomas are complex tumors, often demonstrating components derived from all three embryonic germ layers (37). B, epidermoid cyst shows benign-appearing squamous epithelium lining the cyst, the lumen of which contains layers of keratin. C, the adjacent seminiferous tubules are normal. FISH analysis displays i(12p) in teratoma. Two orange signals for chromosome 12 centromeric probe and three green signals for 12p are present in the cell in the center of the image. Two of the 12p signals are in close proximity to one centromeric signal, with an aggregation pattern (arrow) consistent with i(12p). D, FISH analysis also shows chromosome 12p overrepresentation in teratoma, characterized by two red signals for the chromosome 12 centromere and numerous green signals for the 12p subtelomeric probe. E, FISH pattern typical of epidermoid cyst (disomic chromosome 12 signals).
germ cell tumors, with some evidence that 12p amplification is required for the development of an invasive tumor (6, 24).

Before the dual-color FISH technique became readily available, i(12p) was mainly analyzed by comparative genomic hybridization and/or metaphase chromosome banding methods (30, 31). Using the FISH method, the reported percentage of i(12p)-positive cells ranged from 2% to 8% for a given case (24, 32, 33), with the exception of an early study in which i(12p) was recognized by chromosome centromeric signal size; in that study, 50% to 90% of the cells were reportedly positive for i(12p) (34). In the current study, we used stringent criteria for the identification of i(12p) by interphase dual-color FISH. Only cells that have both the specific signal number and signal spatial relationship are recorded as i(12p)-positive (15, 16). The strict criteria adapted in the current study assured high specificity for the identification of i(12p), but this may also contribute to the limited number of cells being recognized as i(12p)-positive tumor cells. Nonetheless, the percentage of i(12p)-positive cells (2-5%) reported here in mature teratoma is comparable to the percentages reported in the literature cases (32, 35, 36). Because the percentage of cells with recognizable i(12p) signals in germ cell tumors is typically quite low (2-5%), tissues with cell types comparable with those in the studied lesion (e.g., normal squamous epithelium) would serve as optimal controls. FISH assays are best validated when negative controls contain nuclei of similar size to those in the studied sample. This is important because nuclear truncation and overlap-related artifacts are important factors in establishing a negative range for routine assays.

In conclusion, epidermoid cysts are rare, benign testicular neoplasms that are genetically distinct from testicular teratoma because they lack abnormalities in chromosome 12p, including i(12p) and chromosome 12p overrepresentation. Molecular testing for chromosome 12p abnormalities is a rational approach to the distinction of a testicular epidermoid cyst from a testicular teratoma. FISH identification of i(12p) and/or 12p overrepresentation in routinely processed surgical specimens is a useful ancillary diagnostic tool for making this distinction, providing strong support for a diagnosis of teratoma.

**References**


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