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Analysis of ovarian teratomas for isochromosome 12p: evidence supporting a dual histogenetic pathway for teratomatous elements

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Teratomas are the most common germ cell tumor (GCT) of the ovary and include several types with a range of clinical behavior. As in testicular teratomas, they may be benign, malignant or a component of a mixed GCT. In the testis, data support separate pathogeneses for prepubertal and postpubertal teratomas, with derivation of the former from a nontransformed germ cell and the latter from differentiation of a nonteratomatous, malignant GCT. The absence of cytogenetic abnormalities (including isochromosome 12p (i(12p)) in mature ovarian teratomas suggests that they may be analogous to prepubertal testicular teratomas, but there are no data regarding genetic changes in the teratomatous components of ovarian mixed GCTs. We therefore studied the teratomatous components of six mixed GCTs of the ovary using fluorescence *in situ* hybridization (FISH) for i(12p). Six mixed GCTs of the ovary occurred in patients 4–33 years of age; all had teratomatous and yolk sac tumor components and three also contained foci of embryonal carcinoma. Using FISH with 12p telomeric and 12 centromeric probes, five of six (83%) cases had detectable i(12p) in their nonteratomatous components, and four of six (66%) in the teratomatous component. One of the two cases without demonstrable i(12p) in the teratomatous portion of the mixed GCT also did not have identifiable 12p abnormalities in other elements of the mixed GCT. By comparison, five pure, mature ovarian teratomas and three pure, immature ovarian teratomas showed no evidence of either i(12p) or other forms of 12p amplification. These findings support that teratoma in mixed ovarian GCTs has a different pathogenesis compared to pure teratoma of the ovary. Furthermore, the findings of i(12p) in both the teratomatous and nonteratomatous components of ovarian mixed GCTs supports that the teratoma derives from other components, similar to the situation in the testis.
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Ovarian teratomas include several types that exhibit varying clinical behavior; the majority are benign, pure, mature, and cystic.[1,2] Malignant forms include immature teratomas, teratomas with malignant transformation (also designated 'somatic-type tumors' with dermoid cysts), and teratomatous components of mixed germ cell tumors (GCTs). Immature teratomas are much less frequent than their mature counterparts, tend to occur in younger patients, often are solid, and, by definition, have malignant, immature tissue, usually neuroepithelium.[2–5] Teratomas with malignant transformation usually occur in older women, most often contain squamous cell carcinoma, and are rare, accounting for only 1–2% of teratomas.[2,6,7] Mixed GCTs are another rare subset of ovarian GCTs and may be composed of a mixture of mature teratoma, immature teratoma, dysgerminoma, yolk sac tumor, and embryonal carcinoma.[8] To date, there are limited data regarding the origins of these different forms of ovarian GCT, except for mature teratoma where a parthenogenetic-like mechanism is supported based, in part, on homozygosity for polymorphic markers.[9,10] How teratomatous elements develop in mixed ovarian GCTs, however, is currently

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unknown, although there may be parallels with the situation in the testis.

In the testis, data support separate pathogeneses for prepubertal and postpubertal teratoma, namely derivation of the former from a benign, nontransformed germ cell and the latter from differentiation of a nonteratomatous, malignant GCT.[2] There may be comparable differences between the origin of pure ovarian teratomas and the origin of the teratomatous components of ovarian mixed GCTs, but there are no available data regarding cytogenetic changes in the teratomatous components of mixed GCTs of the ovary. It was our hypothesis that these tissues derive from the nonteratomatous components analogous to postpubertal testicular teratomas, and should, therefore, have similar cytogenetic abnormalities. Since ovarian GCTs have been shown to have isochromosome 12p (i(12p)) and 12p amplification,[11,12] the demonstration of i(12p) in the teratomatous components of mixed GCTs of the ovary would support this hypothesis. This study, therefore, examines the origin of ovarian teratomas by using fluorescence *in situ* hybridization (FISH) to look for i(12p) and 12p amplification in the teratomatous components of mixed GCTs, with comparison to findings in mature and immature teratomas.

Materials and methods

Specimens

We analyzed a total of 14 ovarian GCTs using FISH. Six patients whose ages at the time of diagnosis ranged from 4 to 33 years (mean, 18 years) had mixed GCTs. Each of these contained mature teratoma that was admixed with various combinations of other GCT elements including embryonal carcinoma, yolk sac tumor, and immature teratoma (Figure 1) (Table 1). These were compared to eight pure teratomas (five mature (Figure 2), three immature) in patients ranging from 20 to 75 years old (Table 1); none showed 'malignant transformation'.

Fluorescent *In Situ* Hybridization

From each specimen, multiple 4 μm unstained sections were prepared from buffered formalin-fixed, paraffin-embedded tissue blocks. A hematoxylin and eosin stained slide from each case was examined to determine the area of different tumor components.

FISH was performed using previously reported methods.[13] The slides were deparaffinized with two

Table 1 The presence of i(12p) and 12p amplification in mixed germ cell tumors and pure teratomas of the ovary

Case	*Age (years)*	*GCT component*	*12p amplification*	*i(12p)*
1	4	Mature teratoma	–	–
		Embryonal carcinoma	+	+
		Yolk sac tumor	+	+
2	10	Mature teratoma	–	–
		Immature teratoma	+	+
		Yolk sac tumor	–	+
3	33	Mature teratoma	–	–
		Embryonal carcinoma	–	–
		Yolk sac tumor	–	–
4	24	Mature teratoma	–	+
		Yolk sac tumor	+	+
5	14	Teratoma	–	+
		Embryonal carcinoma	–	–
		Yolk sac tumor	–	+
6	23	Teratoma	–	+
		Yolk sac tumor	–	+
7	20	Mature teratoma	–	–
8	36	Mature teratoma	–	–
9	60	Mature teratoma	–	–
10	75	Mature teratoma	–	–
11	47	Mature teratoma	–	–
12	21	Immature teratoma	–	–
13	30	Immature teratoma	–	–
14	32	Immature teratoma	–	–

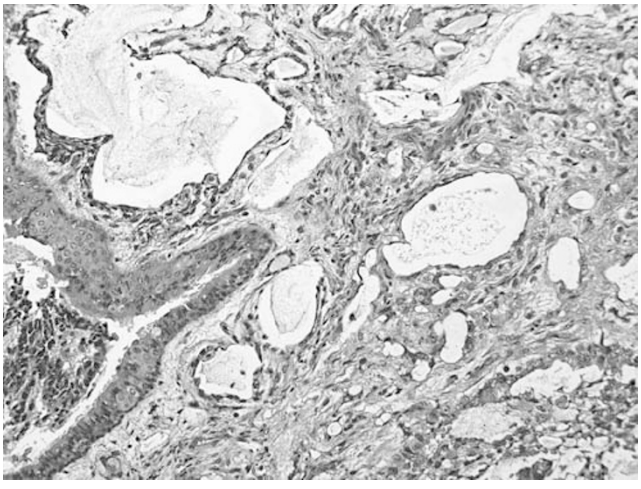

Figure 1 Ovarian mixed germ cell tumor showing a teratomatous component of respiratory epithelium (left) with yolk sac tumor.

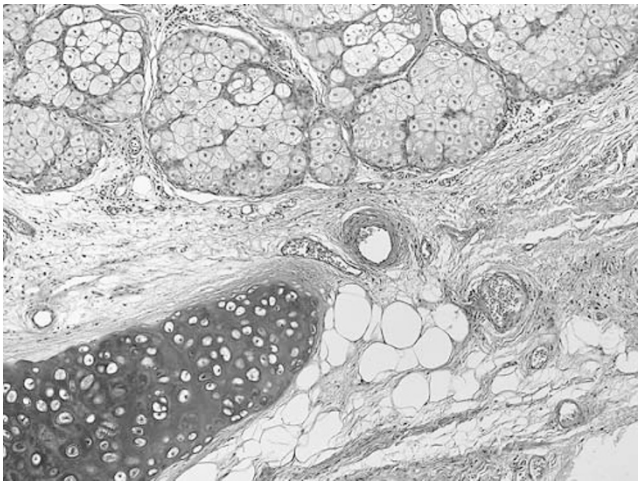

Figure 2 Mature teratoma with sebaceous glands and cartilage.

washes of xylene, 15 min for each and were subsequently washed twice with absolute ethanol, 10 min each. The slides were rinsed with distilled water for 3 min and further washed with 2 × standard saline citrate (SSC) for 5 min and allowed to air dry in a hood. Next, the slides were treated in 0.1 mM citric buffer (pH 6.0) (Zymed, CA, USA) at 95°C for 10 min, rinsed in distilled water for 3 min, followed by a wash of 2 × SSC 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 then washed with distilled water for 3 min and further washed with 2 × SSC for 5 min and air-dried. 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 reduced light conditions, and each slide was covered with a 22 × 22 cover slip and the edges were 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 overnight.

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

The slides were examined using a Zeiss Axioplan 2 microscope (Zeiss, 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). For each case up to 20 fields were captured under a 100 × oil objective. 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 artefacts. Criteria for the signal detection were described previously.[14–16] Fifty to 200 cells were counted for each of the components. Classic seminoma of the testis was used as a positive control.

Results

Either i(12p) (Figure 3) or 12p amplification (Figure 4) was observed in five of 14 cases examined (Table 1). None of the pure mature or immature teratomas displayed either i(12p) or 12p amplification as defined by a 12p/12 centromeric ratio of 1.5 or greater. In the pure mature and immature teratomas, the 12p portion of the chromosome was present in a ratio of 0.96–1.06 times the centromeric portion (mean, 1). Four of six mixed GCTs had i(12p) in the teratomatous components and five of the same cases had i(12p) in the nonteratomatous component (Table 1). In the teratomatous components of the mixed GCTs, the 12p portion of the chromosome was present in a ratio of 1.01 to 1.26 times the centromeric portion (mean, 1.2). In the nonteratomatous components of the malignant GCTs, the 12p portion of the chromosome was present in a ratio of 1.03–1.68 times the centromeric portion (mean, 1.3). One of two cases that did not display 12p abnormalities in the teratomatous portion of the mixed GCT also did not demonstrate them in other elements of the mixed GCT. In the teratomatous areas of the sixth case, the foci of teratoma had 12p amplified to 1.26 times the centromeric portion, which was below the cutoff value for amplification but well above the range found in the pure teratomas (0.96–1.06). The positive control did show i(12p) and evidence of 12p amplification, with a 12p to centromeric signal ratio of 1.52.

Discussion

Study of prepubertal and postpubertal testicular teratomas supports their separate pathogenesis, with the former deriving from a benign germ cell and the latter from a nonteratomatous malignant GCT.[2,17] In this model, virtually all adult testicular teratomas are initially a component of a mixed GCT, and the occasional pure teratoma of the adult testis mostly represents the persistence of teratoma with spontaneous regression of other GCT types. Evidence supporting the origin of postpubertal testicular teratomas and the teratomatous components of mixed GCTs of the testis from malignant germ cells includes their association with intratubular germ cell neoplasia, unclassified (IGCNU),[18] the presence of i(12p) and of chromosome 12p amplification in both teratomatous and nonteratomatous components of postpubertal GCTs,[19–23] the presence of aneuploidy in postpubertal teratomas,[22,24] the similarities in allelic losses between mature teratoma and other components of malignant mixed GCTs of the testis,[25] the malignant behavior of postpubertal testicular teratomas,[26–28] and the atypical cytologic appearance of some postpubertal testicular teratomas.[2,17] Conversely, evidence supporting the origin of prepubertal testicular teratomas from benign germ cells includes a lack of chromosome 12p amplification,[29] a generally diploid, 46 XY karyotype,[30] normal findings on comparative genomic hybridization studies,[31] and lack of IGCNU.[18] It is also well established that most ovarian teratomas derive from

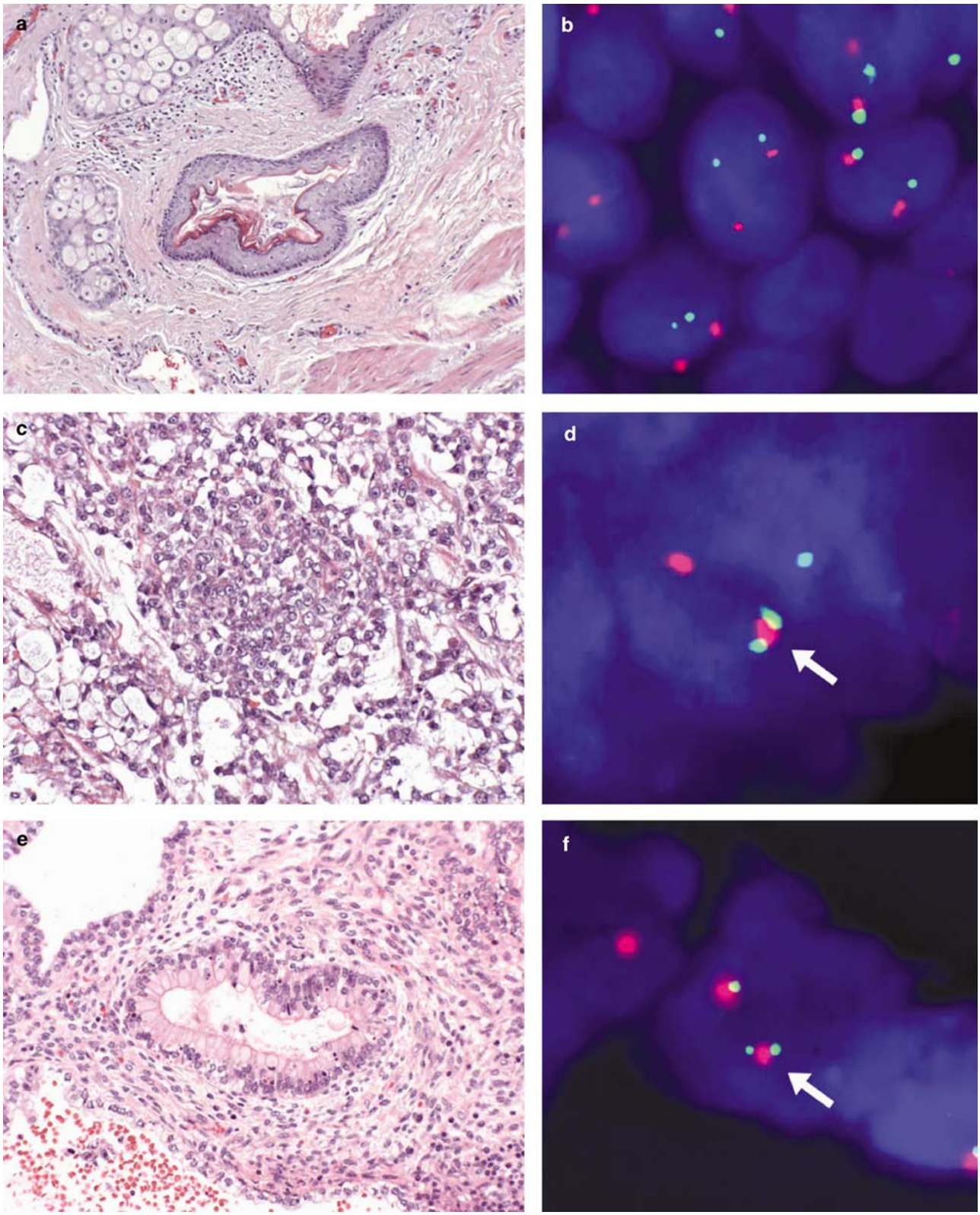

Figure 3 (**a**) Mature teratoma. (**b**) Correlating FISH of the tumor shown in (**a**) with normally distributed 12p telomere (green) and 12 centromere (red) signals. (**c**) Yolk sac tumor component from a mixed ovarian germ cell. (**d**) Correlating FISH of the tumor shown in (**c**) with close juxtaposition of 12p telomere signals (green) with 12 centromere signal (red), indicating i(12p) (arrow). (**e**) Teratoma in mixed germ cell tumor. (**f**) Correlating FISH of the teratoma shown in (**e**) demonstrating i(12p).

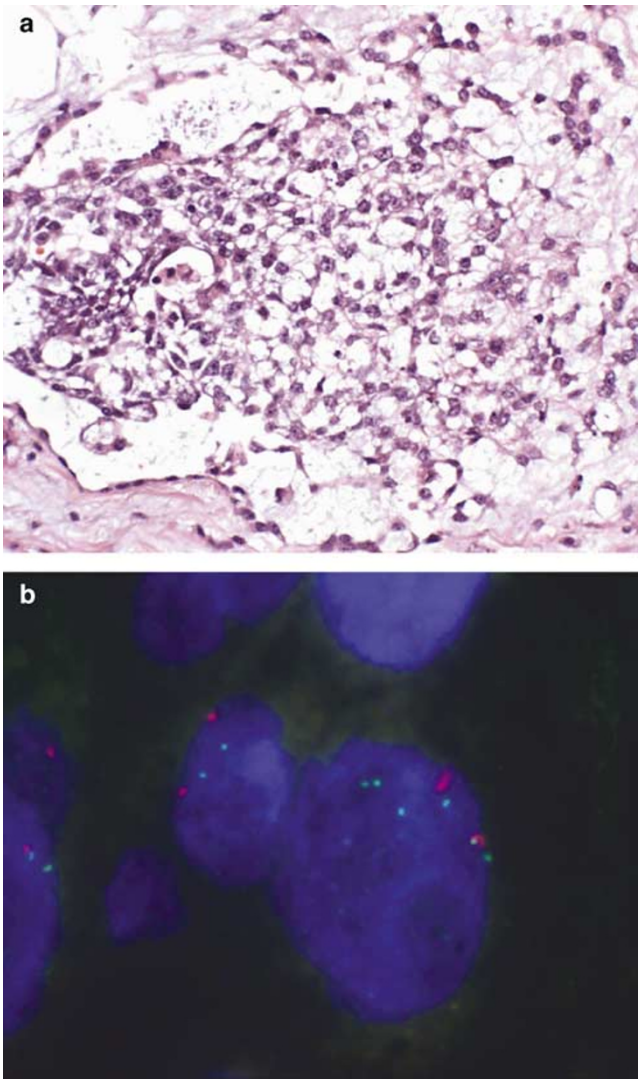

Figure 4 (**a**) Yolk sac tumor component of a mixed germ cell tumor. (**b**) Correlating FISH of the tumor shown in (**a**) demonstrating 12p amplification (green signals—12p telomere; red signals—12 centromere).

benign germ cells in a parthenogenetic-like fashion based on their cytogenetics,[11,12] ploidy,[32] and molecular biologic findings.[10] In contrast to most testicular teratomas, they lack karyotypic abnormalities, including i(12p), but have a normal, 46, XX karyotype,[11,33] contain a diploid amount of DNA,[32] and show a homozygous pattern for polymorphic markers.[9,10] The latter observation suggests a parthenogenetic-type origin from a germ cell between meiosis I and meiosis II. A second ovarian pathway, analogous to the predominant pathway in the testis, is less well founded. We hypothesized that the teratomatous components of mixed GCTs of the ovary would have a pathogenesis similar to that of postpubertal testicular teratoma[2,17] and anticipated that they would show 12p abnormalities similar to those found in nonteratomatous malignant GCTs of the ovary.

Our results support that the teratomatous components of mixed GCTs of the ovary have a different pathogenesis from pure mature and immature ovarian teratomas and likely derive from the nonteratomatous components of such tumors. Of cases of ovarian mixed GCT, 83% had i(12p) in their nonteratomatous components and 66% in their teratomatous components, whereas all cases of pure mature cystic teratoma and immature teratoma lacked i(12p) or other evidence of 12p amplification. These findings parallel the presence of i(12p) and other forms of 12p amplification in postpubertal testicular teratomas[19–23] and the absence of such genetic findings in prepubertal testicular teratomas.[29–31] Additionally, the finding of 12p amplification in both the teratomatous and nonteratomatous components of ovarian mixed GCTs supports the origin of the former from the latter.

The findings of this study are in general agreement with those of others who examined the genetics of ovarian GCTs. Baker *et al*[32] found that nine of 11 immature teratomas were diploid (with the two immature teratomas classified as aneuploid later being found to contain other GCT elements) and the majority of other GCT subtypes were aneuploid, with a mean DNA index of 1.85, the latter result similar to that found in adult testicular GCTs. Kraggerud *et al*[11] examined 25 ovarian GCTs, finding gains of 12p in 67% of dygerminomas and in 75% of yolk sac tumors; however, no gain of 12p was found in 11 immature teratomas. A single mixed ovarian GCT showed similar chromosomal abnormalities (including two copies of i(12p)) to those of adult testicular GCTs.[12] All of these studies collectively support a similar histogenesis for malignant ovarian GCTs and testicular GCTs, with a separate mechanism for the pure teratomas of the ovary. These results are in line with our findings.

In conclusion, we identified the frequent presence of i(12p) in the teratomatous and nonteratomatous components of ovarian mixed GCTs and its absence, as well as other evidence of 12p amplification, in mature cystic ovarian teratomas and in immature ovarian teratomas. The data therefore support two pathogenetic models for ovarian teratomas: the major pathway, similar to that of prepubertal testicular teratomas, is followed by pure mature and immature teratomas and represents derivation from nontransformed germ cells; the second, minor pathway, similar to that of postpubertal testicular teratomas, is followed by the teratomatous components of mixed GCTs, with their derivation from associated nonteratomatous components.

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