Sclerosing stromal tumors of the ovary: a clinicopathologic, immunohistochemical and cytogenetic analysis of three cases

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Summary

Sclerosing stromal tumors (SSTs) are uncommon ovarian neoplasms of the sex cord-stromal category, that usually occur below 30 years of age. In the present study three cases of SSTs, diagnosed during the last eight years in our hospital, were examined immunohistochemically with stains for estrogen receptors, α and β, progesterone receptors, and stains for markers that have been reported to be of use in the diagnosis of sex cord-stromal tumors. They were also examined by fluoresence in situ hybridization (FISH) for the presence of trisomy 12 and 7. Positivity for ERβ was observed in a significantly larger number of cells than ERα. Positivity for calretinin and A103 was observed in tumor cells. In two cases 20-30% of the nuclei showed trisomy 12. No aberration of chromosome 7 was detected. The finding of increased ERβ expression needs further investigation.

Key words: Sclerosing stromal tumor; Estrogen receptor α; Estrogen receptor β; Progesterone receptor; Chromosome 12.

Introduction

Sclerosing stromal tumors (SSTs) are uncommon ovarian neoplasms of the sex cord-stromal category, first described by Chalvardjian and Scully in 1973 [1]. They comprise 2.5% of sex cord-stromal ovarian tumors [2] and the majority of them, about 80% of cases, occur below 30 years of age. Like other sex cord-stromal tumors of the ovary, they may pose problems in the differential diagnosis; immunohistochemistry can be of help in these cases.

It has been suggested that SSTs are derived from pluripotent immature stromal cells of the ovarian cortex [3] or from perifollicular myoid stromal cells that exist in the theca externa [4]. Positivity for steroid hormone receptors, predominantly progesterone receptors, has been described in some [5, 6] but not all reports [7, 8].

In recent years several authors have examined the role of estrogen receptor β in epithelial tumors of the female genital tract [9-11], but there are few reports concerning sex cord-stromal tumors [12].

Trisomy 12 is a relatively common chromosomal aberration in neoplasms of the granulosa-stromal cell group, while it is not a feature of Sertoli-stromal cell tumors [13-17]. The percentage varies in different studies, probably due to differences in techniques and small numbers of cases. Trisomy 12 has been reported to occur in SSTs [5], although reports concerning cytogenetic analysis of this group of tumors are limited.

In the present study cases of SST from our material were examined immunohistochemically with stains for estrogen receptors, α and β (ERα and ERβ), progesterone receptors (PR), and with stains for two markers that have been reported to be of use in the diagnosis of sex cord-stromal tumors: calretinin, a calcium-binding protein, and A103, an antibody to Melan A [18-21]. They have also been examined by fluoresence in situ hybridization (FISH) for the presence of trisomy 12 and 7.

Materials and Methods

Three cases of SSTs diagnosed during the last eight years in our hospital were examined. The number of tissue blocks ranged from five to eight and the most representative two or three were used for the study. The tumors were immunostained for estrogen receptor (6F11, Novocastra, 1:40), estrogen receptor β (polyclonal 385P, Biogenex, 1:50), progesterone receptor (PgR 636, DAKO, 1:40), calretinin (5A5, Novocastra, 1:100), A103 (Novocastra, 1:50). Immunostains were performed on an automated staining system (Biogenex). Microwave antigen retrieval in citrate buffer, pH 6.0, was performed in all cases. Positive and negative controls were used for each antibody. Staining was scored as negative (–), when up to 5% of the cells showed weak staining, or positive, graded from 1+ to 3+, depending on the extent and intensity of staining.

α-satellite probes (D12_3 and D7Z1, Appligene-Oncor) were used for FISH on serial 4 μ-thick sections. Hybridization was carried out according to the manufacturer's published protocol. Two hundred nuclei were evaluated per section. A signal gain count of > 10% of nuclei was the threshold for evaluation of trisomy, based on the low percentage of trisomic nuclei in non-neoplastic ovarian tissue of our material, as well as that of other reports [15].

Four sections of nonneoplastic ovarian tissue from hysterectomies performed for uterine lesions were included for comparison. Sections from 12 cases of carcinomas, six ovarian and six metastatic to the ovary from the gastrointestinal tract, were included in the immunohistochemical study of calretinin and A103.

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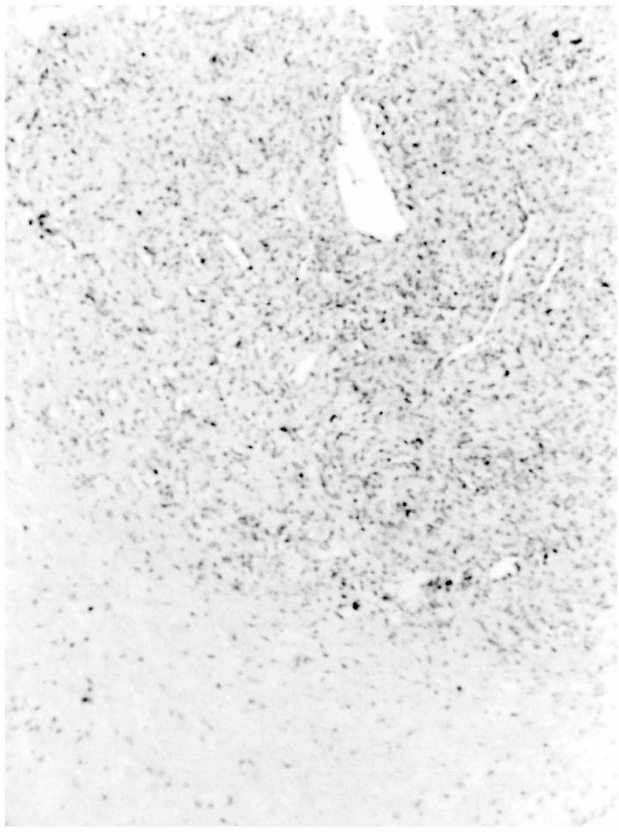

Figure 1. — Expression of ERβ (original magnification x 200).

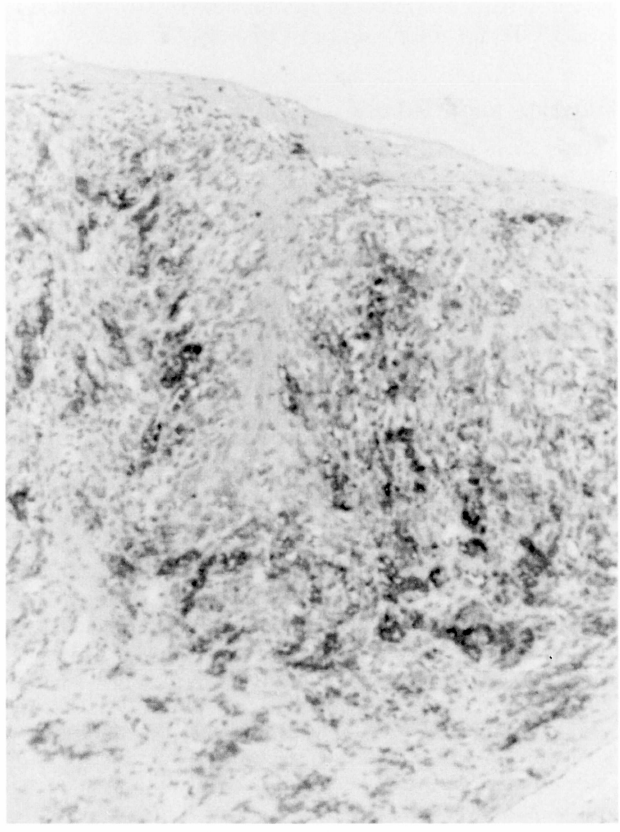

Figure 2. — Expression of calretinin in SST (original magnification x 200).

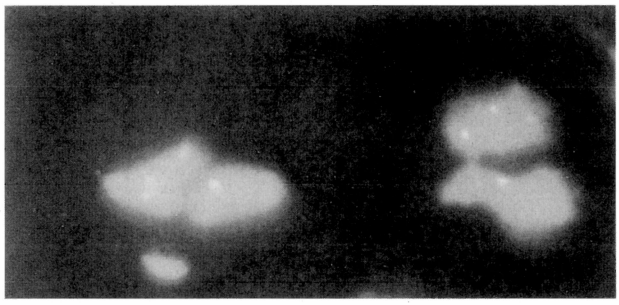

Figure 3. — Trisomic nuclei (original magnification x 1,000).

Results

The clinical and gross features are summarized in Table 1. The age of the patients varied between 15 and 23 years (mean: 18.3 years). In one case the patient presented with menstrual irregularities. In another case the tumor was an incidental finding, as it was located in the wall of a large endometriotic ovarian cyst. The follow-up period ranged from two to eight years and was uneventful.

Grossly the tumors were solid, lobulated, white or yellowish on the cut surface. Histologically, all three tumors exhibited a pseudolobular pattern with a variety of cell types characteristic of SSTs. In case 1 a significant number of smooth muscle cells, positive for smooth muscle actin and desmin, was observed microscopically. Groups of these cells occupied significant areas of the tumor, especially in the edematous areas between cellular nodules.

The results of immunohistochemistry and FISH are presented in Table 2 (Figures 1-3).

All SSTs exhibited granular cytoplasmic positivity for A103 in the rounded or polyhedral cells of the cellular areas. Two cases showed strong positivity for calretinin, predominantly in the same areas. Two carcinomas showed weak positivity for calretinin mostly cytoplasmic. Positivity for ERβ was observed in 40% to 75% of the nuclei in the SSTs. Positivity for ERα was observed in fewer cells than positivity for ERβ, up to 20% of the tumor cells, usually in places, and was weak to moderate in intensity. Positivity for PR was observed in 30-40% of the tumor cells.

Twenty to thirty percent of the nuclei in two cases showed trisomy 12. Only 0-3% of the nuclei appeared trisomic in sections from normal ovarian tissue. No significant aberrations of chromosome 7 were detected.

Table 1. — *Case profiles.*

Case	Age	Location	Presentation	Size	Gross appearance
1	23	L	Incidental finding[a]	4 cm	Solid
2	17	L	MI	4.5 cm	Solid
3	15	R	LAM	6 cm	Solid

[a]The tumor was located in the wall of an endometriotic ovarian cyst; MI: menstrual irregularities; LAM: lower abdominal mass.

Table 2. — *Results of FISH and immunohistochemical study.*

Case	Trisomy 12	Calretinin	A103	ERα	ERβ	PR
1	30%	3+	2+	1+	3+	2+
2	20%	–	2+	1+	2+	2+
3	4%	2+	2+	–	2+	1+

Discussion

Positivity for estrogen (ERα) and progesterone receptors has been described in SSTs by Kawauchi *et al.* [5], who considered this as an indication of autocrine-type stimulation. This type of stimulation might lead to the expression of vascular permeability factor/vascular endothelial growth factor (VPF/VEGF), thus inducing the prominent vasculature and edema, which histologically characterize SSTs. The authors reported that the number of PR-positive tumor cells was greater and more widely dispersed than ER-positive tumor cells. Lifschitz *et al.* [6] also reported immunohistochemical positivity for PR but not ER in one case examined, while Stylianidou *et al.* [7] had negative results. Marelli *et al.* [8] reported a low steroid receptor content in one tumor examined. In our cases positivity for ERβ was observed in a significantly larger number of cells than ERα. High expression of ERβ has also been described in granulosa cell tumors [12, 22]. Although the exact roles of estradiol-17β and its receptors in serving the different actions of estrogen in the ovary have not been completely clarified, ERβ may comprise a part of an autocrine loop and might be of pathogenetic significance in SSTs.

Recent reports describing immunohistochemical results of new antibodies applied on sex cord-stromal tumors usually include only small numbers of SSTs, due to the rarity of the latter neoplasm. The antibody A103 recognizes an antigen, designated melan-A or MART-1, expressed by normal melanocytes and melanocytic proliferations. A103 also labels steroid-producing cells, adrenocortical neoplasms and sex cord-stromal tumors [20, 21, 23]. Positivity in the latter appears to be due to cross-reactivity with another epitope rather than true expression of Melan-A antigen [20]. Stewart *et al.* [21] reported positivity for A103 in two cases of SSTs examined and our results support this observation. The extent of staining in sex cord stromal tumors varies. Positivity for A103 may be rarely observed in ovarian carcinomas [23], but has not been found in metastatic tumors of the ovary [21], although occasional immunoreactive stromal cells may be present. In our study we also observed absence of staining in tumor cells of six metastatic carcinomas from the gastrointestinal tract, which were included in the study since an important differential diagnosis of SSTs is from the Krukenberg tumor. It should be noted that the steroidogenic capability of SSTs has been repeatedly examined in the literature with different results, since the first description of these tumors by Chalvardjian and Scully [1]. The above authors did not find convincing evidence of hormonal activity, although in some subsequent reports evidence suggestive of hormonal activity has been described [24-26].

Immunostain for calretinin, a 29kDa calcium-binding protein, useful in the diagnosis of mesotheliomas, has been reported to be often positive in sex cord-stromal ovarian tumors [18, 27, 28], probably related to secretion of steroids or coupled to other cellular metabolic processes [29]. A recent study has shown the high sensitivity of this marker in the diagnosis of sex cord-stromal neoplasms, although its specificity was lower than that of α-inhibin [29]. The above study included two cases of SSTs which were positive for calretinin. McCluggage and Maxwell [18] reported positivity in one SST examined. In another report by Tiltman and Haffajee [26] positivity was observed in 11 cases examined but it was described as less intense than positivity for αGST, another marker examined by the authors, related to steroidogenesis. Although positivity may also be observed in a small percentage of adenocarcinomas [18, 27, 30, 31], as in the present study, it is usually limited in extent and/or weak. It is noteworthy that the only negative SST in our study was the oldest case and the influence of difference in fixation or storage could not be excluded.

Concerning cytogenetic analysis of SSTs, Lopes *et al.* [32] described a case of SST with monosomy of chromosome 16. Kawauchi *et al.* [5] reported the presence of trisomy 12 in 13-21% of nuclei in all five SSTs examined, while for chromosomes 16 and 17 they did not observe significant aberrations. Since in their criteria the threshold for trisomy was 20% of the nuclei, the authors considered only one SST to have chromosome 12 trisomy.

Isolated trisomy 12 appears to be a relatively common finding in benign and mesenchymal tumors, although it is not restricted to these tumor types. A proportion of ovarian fibrothecomas, granulosa cell tumors and fibrosarcomas have been reported to exhibit trisomy 12 and rarely tetrasomy 12 [13-16]. Its common presence may suggest a degree of similarity in the evolution of histologically different neoplasms of the granulosa-stromal cell group, in contrast to Sertoli-Leydig cell tumors, which exhibit other cytogenetic aberrations [17, 33]. The presence of trisomy 12 in only a percentage of the nuclei in the cases examined in the present study, as has been observed in other tumors of the granulosa-stromal cell group, needs further elucidation.

In summary, trisomy 12 is a common finding in ovarian SSTs, as has been reported for other neoplasms of the granulosa-stromal cell group. Although the number of our cases is small, it appears from the above results that antibodies for calretinin and melan A can be of help, as part of a panel, in the differential diagnosis of SSTs from carcinomas. The finding of increased ERβ expression and its possible role needs further investigation.

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