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ORIGINAL ARTICLE

Sertoli–Leydig cell tumours of the ovary and testis: a CGH and FISH study

I. Verdorfer · D. Horst · A. Höllrigl · H. Rogatsch · G. Mikuz

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Abstract We present two malignant cases of Sertoli–Leydig cell tumours (SLCT) of the testis and one ovarian SLCT with benign behaviour. The DNA copy number changes affected chromosome 1, 8, 9p, 10, 11, 12, 16, 19, 22 and X. The present study is the first molecular–cytogenetic analysis of Sertoli–Leydig cell tumours of the testis.

Keywords Comparative genomic hybridisation · Fluorescence in situ hybridisation · Sertoli–Leydig cell tumours · Sex-cord stromal tumours

Introduction

The Sertoli–Leydig cell tumours (SLCT) are exceptionally rare sex-cord stromal tumours of the testis [10, 18]. There are, however, some few cytogenetic studies of SLCT of the ovary, which revealed genetic aberrations affecting the sex chromosome [1, 3, 4]. Trisomy 8 was detected in a metastasizing ovarian SLCT as sole karyotypic aberration [7]. A case of virilising ovarian SLCT in a 14-year old black girl showed chromosomal rearrangements of chromosome 5, 18, trisomy 6 and 12 [15]. Cytogenetic studies of such tumours located in the testis have never been reported. We analysed therefore the chromosomal aberrations of two testicular and one ovarian SLCT.

I. Verdorfer (✉) · D. Horst · A. Höllrigl · G. Mikuz
Institute of Pathology, Medical University of Innsbruck,
Muellerstrasse 44,
6020 Innsbruck, Austria
e-mail: Irmgard.Verdorfer@i-med.ac.at

H. Rogatsch
Institute of Pathology, LKH Klagenfurt,
9020 Klagenfurt, Austria

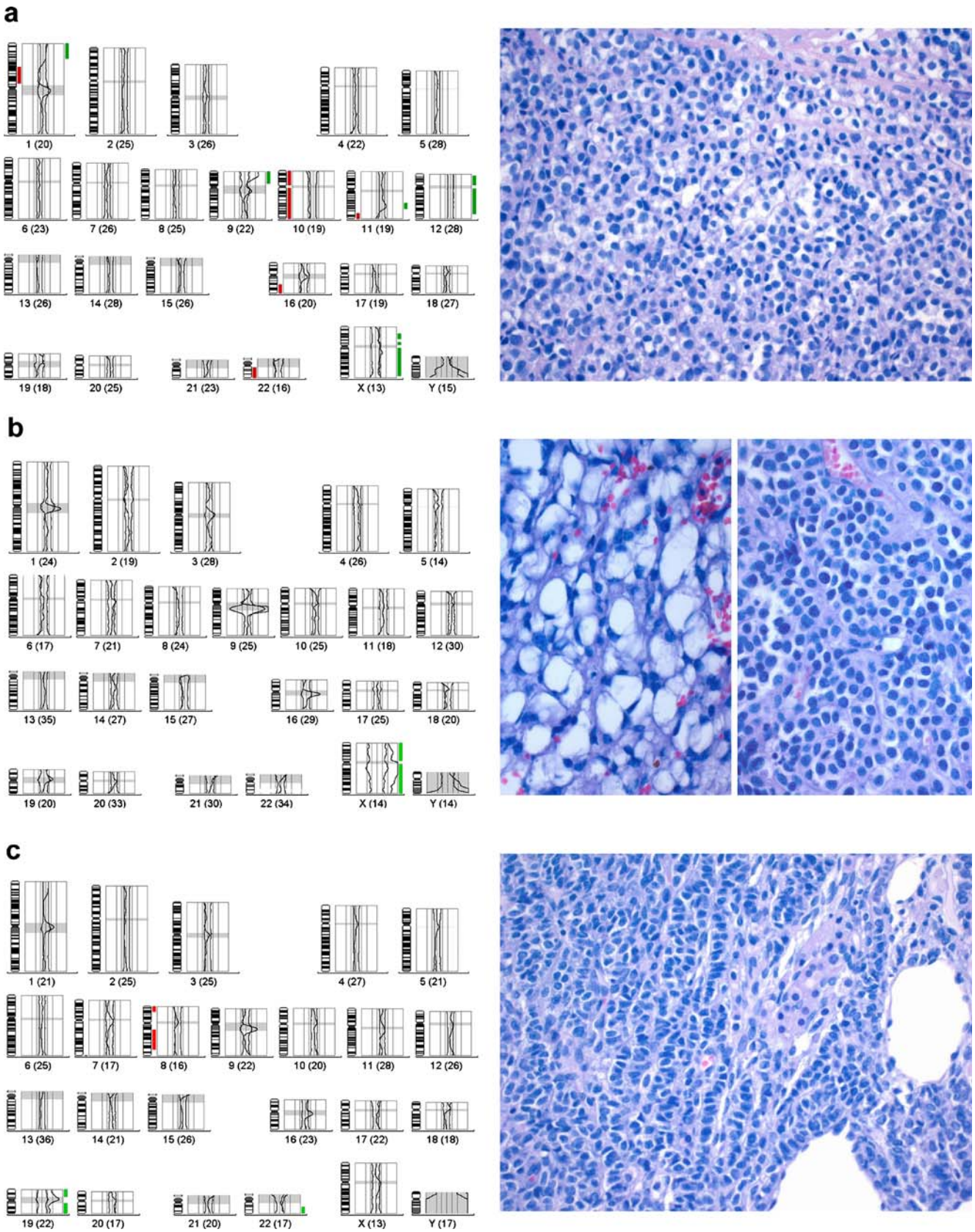
Materials and methods

Tumour specimens and immunohistochemistry

Three SLCT, retrieved from the files of the Institute of Pathology Innsbruck (Medical University Innsbruck, Austria) were examined. Tissue sections were stained with haematoxylin and eosin, profiled immunohistochemically and classified according to the WHO classification of testicular tumours [12].

Comparative genomic hybridisation

Comparative genomic hybridisation (CGH) analysis was performed as previously described [17]. Briefly, tumour DNA and reference DNA were labelled with biotin-16-dUTP and digoxigenin-11-dUTP, respectively, by nick translation using a commercially available nick translation kit (Roche, Mannheim, Germany). The probe mixture of 500 ng tumour DNA, 500 ng reference DNA, 20 μg human Cot-1 DNA (Gibco BRL, Life Technologies, Germany) and 10 μg salmon sperm DNA (Eppendorf, Germany) was denatured for 5 min at 75°C and pre-annealed for 20 min at 37°C. The probe mixture was hybridised on a normal, denatured lymphocyte metaphase slide and hybridised 3 days in a humidified chamber. For detection, the slides were stained with avidin–fluorescein isothiocyanate (Vector Labs, Burlingame, CA, USA) and with anti-digoxigenin–rhodamin (Roche, Mannheim, Germany), followed by counter-staining with 4,6-diamino-2-phenylindole (DAPI; Serva, Germany). The slides were evaluated using a fluorescence microscope (Zeiss Axioplan) equipped with a CCD camera (JAI M300), specific filter sets and CGH software (ISIS, MetaSystems, Germany).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◀ **Fig. 1** CGH profiles (*left*) and the corresponding histology of the tumours (*right*, H&E, ×40). **a** Case 1 (lung metastasis) showed alterations on chromosome 1p, 9p, 10, 11q, 12, 16q, 22q and X. Histomorphological features were nested tumour formations, eosinophilic cytoplasm, oval to spindle-shaped nuclei. **b** Case 2 with gain of chromosome X. The malignant tumour showed morphologies of a lipid-rich Sertoli-cell component (*left*) and a nested Leydig-cell component (*right*). **c** Case 3 with partial loss of chromosome 8 and gain on chromosome 19 and 22. The benign tumour showed Sertoli-cells in a trabecular arrangement and islands of Leydig-cells

Gains or losses were calculated as significant by the evaluation software if fluorescence ratio showed borderline values of 0.8 and 1.25, respectively. Pericentromeric, heterochromatic regions, telomeric regions and chromosome Y were excluded from the evaluation.

Fluorescence in situ hybridisation

To evaluate the CGH results, fluorescence in situ hybridisation (FISH) on paraffin-embedded sections was performed according to the manufacturer's protocol. Tissue sections (5 μm) were mounted on adhesion slides and dried at 70°C for 30 min. The inter-phase preparation and hybridisation were performed according to the protocol of the manufacturer (Abbott/Vysis, Downers Grove, IL, USA). DNA probes for the centromeric region for chromosome X/Y, 8, two LSI probes for 9p21 and 22q11.2 (Abbott/Vysis) and a locus specific probe on 12q14-15 (YAC probe 745a10, kindly provided by Dr. Kalscheuer, Max Planck Institute, Berlin) were used. At least 100 nuclei in each case were examined.

Results

Pathologic and immunohistochemical findings

Microscopically case 1 (lung metastasis lung metastasis from a 66-year-old male patient) showed uniform cells, arranged in nested tumour formations, with eosinophilic cytoplasm, oval to spindle-shaped cells with not overtly prominent nuclei and an increased mitotic rate (26 mitotic figures/10 HPF). Only the Leydig cell component showed formation of metastases (Fig. 1a, right). The second malignant tumour of the testis from a 75-year-old patient (case 2) showed morphologies of lipid-rich Sertoli-cell components (Fig. 1b, left) and a nested Leydig-cell component (Fig. 1b, right). The tumour showed vascular invasion and an increased mitotic rate (33 mitotic figures/10 HPF).

The cells of case 3 (ovarian SLCT from a 74-year-old woman) appeared bland and uniform; the benign tumour showed Sertoli cells in a trabecular arrangement and islands of Leydig cells. Immunohistochemical analysis showed positivity of the Leydig-cell component of the benign tumour (case 3) for α-inhibin and calretinin whilst both components stained negative for Melan A (A 103). Both malignant tumours (case 1 and 2) were completely negative for these markers (Table 1).

CGH and FISH analysis

All analysed cases showed chromosomal imbalances. Case 1 showed gains and losses involving 10 chromosomes or chromosomal regions. The second case showed gain on the entire chromosome X as sole aberration. The third case was with loss of the entire chromosome 8 and gain of chromosome 19 and 22. CGH profiles and histomorphological features of the tumours are shown in Fig. 1. The FISH results were in agreement with the CGH data. Results of CGH and FISH data are given in Table 2.

Discussion

Sertoli–Leydig tumours of the ovary and the testis are scarce neoplasm [9] exceptionally rare in the testis [10, 18]. Only few cytogenetic studies of ovarian sex-cord stromal tumours and no chromosomal analysis in SLCT of the testis are reported in the literature. The present CGH analysis in two male patients with SLCT is the first study of genetic aberrations in these tumours, affecting entire chromosomes (or chromosome regions) on 1p, 8, 9p, 11q, 12, 16q, 19, 22 X.

Trisomy 8 has been described in fibrosarcomas of the ovary as one of several aberrations [16]. Manegold et al. [7] found trisomy 8 as sole karyotypic aberration in an ovarian metastasizing SLCT. These findings are in contrast to our CGH data. One of the three analysed tumours showed loss and no gain of chromosome 8 (Fig. 1c, left) as part of other chromosomal imbalances.

Simple numeric changes, patriculary trisomy 12 and monosomy 22, have been described as recurrent findings in ovarian sex-cord stromal tumours [13], including granu-

Table 1 Patients' profile

Case	Age/Sex	Clinical behaviour	IHC profile		
			α-inhibin	Melan A	Calretinin
1[a]	66 years/m	M	–	–	–
2	75 years/m	M	–	–	–
3	74 years/f	B	L+, S–	–	L+, S–

B Benign, *M* malignant, *m* male, *f* female, + positive staining, – negative staining, *L* Leydig-cell component, *S* Sertoli-cell component
[a] Lung metastasis

Table 2 Results of CGH and FISH

Case	DNA copy number changes		FISH results of target anomalies [% analysed nuclei]
	Losses	Gains	
1[a]	1p31.3p12, 10	1pterp32.3, 9p	3–9 (mainly 3–4) copies of 9p21 [44]
	11q23.3qter	11q14.2q23.1	3–4 copies of 12q14q15 [50]
	16q21qter, 22	12, X[b]	2–5 copies of X [38] and 2–3 copies of Y [19], mainly XXY or XXYY
2		X	2–3 copies of X and loss of Y [80], (mainly XX)
3	8[b]	19, 22q	3 to 4 copies of 22q11.2 [30]

[a] Lung metastasis
[b] Imbalance with a clear shift, but the CGH profile shows only a partially significant imbalance (partially reaching the threshold)

losa-stromal cell tumours [14], fibrothecomas [16], in sclerosing stromal tumours [5] and ovarian tumours [6, 11]. Also, the CGH and interphase FISH study by Mayr et al. [8] on ovarian granulosa cell tumours corroborate these previous reports of the prevalence of trisomy and monosomy 22. Truss et al. [15] found trisomy 12 in a virilizing ovarian SLCT as part of a number of other aberrations, including an overexpression of the BCL-2 gene, caused by rearrangement of chromosome 18 with a clonal karyotype 48XX,der(5)t(5;18)(p13;q12),+6,+12,der(18)r(5;18)(p15.3p13;p11.3q12).

We found gain of entire chromosome 12 and monosomy 22 in case 1 by CGH. However, a discrepant result regarding chromosome 22 was seen in case 3: not monosomy 22 but gain of chromosome 22 was detected. The FISH analysis confirmed this, showing three to four signals for 22q11.2 in 30% of the analysed nuclei (Table 2). De Giorgi et al. [1] presented a case of ovarian SLCT with endometrioid-like yolk sac tumour in a 26-year-old patient with a 46,XX karyotype with Y-chromosomal material insertion into chromosome 1. A well-differentiated SLCT of the ovary in a 48-old-year Japanese woman with symptoms of virilisation and a 45,X/46XX/47XXX karyotype is reported by Hitosugi et al. [3]. Another Sertoli–Leydig cell tumour in a woman with XO/XX/XXX-mosaicism is reported by Glaser et al. [2]. We provide the first molecular-cytogenetic analysis of testicular SLCT, identifying gain of chromosome X in both analysed cases (case 1 and 2) by CGH. FISH analysis showed two to three copies of chromosome X in both cases; in one of them, loss of chromosome Y was observed in 80% of the analysed nuclei.

In conclusion, aberrations on chromosome 12 and on sex chromosomes suggest associations with oncogenetic mechanisms in these groups of neoplasms. However, the characterisation of SLCT in this study represents an additional contribution to the poorly explored SLCT of the ovary and testis.

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