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Definition of a New Entity of Malignant Extragonadal Germ Cell Tumors

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Two malignant extragonadal germ cell tumors are reported, histologically classified as immature teratomas, having pseudo-diploid karyotypes with complex structural rearrangements but lacking isochromosome 12p or other rearrangements involving 12p. The absence of 12p material in structural rearrangements was confirmed by chromosome painting. In the two tumors the following common chromosomal breakpoints were found: 6p21, 6p22, 6q23, and 11q13. Exactly the same chromosomal regions, 6p22::6q23 and 6p21::11q13, were involved in fusions. The two tumors belong to a new entity of extragonadal immature teratomas of adults which may be located in the retroperitoneum and posterior mediastinum and are prone to blood borne metastasis. Genes Chromosom Cancer 12:8-15 (1995). © 1995 Wiley-Liss, Inc.

INTRODUCTION

Human germ cell tumors (GCTs) are a heterogeneous group of neoplasms located in the testis, the ovary, and in extragonadal sites. Their pathogenesis, histological composition, cytogenetics, ploidy, and degree of malignancy differ, depending on the anatomical site of the tumor and the patient’s sex and age (Oosterhuis et al., 1990).

Extragonadal GCTs by themselves are a heterogeneous group of rare neoplasms. They occur in the midline of the body (pineal region, hypothalamic region, anterior and posterior mediastinum, retroperitoneum, and sacral area), but also away from the midline (e.g., orbit, neck, stomach, placenta) (Oosterhuis et al., 1990).

In adult males one has to exclude the possibility of a metastasis of a testicular GCT before assuming that the tumor is an extragonadal GCT, particularly in the case of retroperitoneal tumors (Daugaard et al., 1987).

We report two pseudo-diploid extragonadal GCTs with common abnormalities of chromosomes 6 and 11. The two tumors seem to belong to a separate entity which is not only defined cytogenetically, but also by its pathological characteristics and clinical behaviour. Our cytogenetic data and published reports allow a cytogenetic classification of malignant extragonadal GCTs. It is possible that the cytogenetically different tumor types originate from different cell types and by different pathogenesis.

CASE REPORT

Case I

A 65-year-old man presented with a soft tissue mass in his left thigh, which he had first noticed 6 weeks before. A biopsy revealed tumor tissue with the histological appearance of immature teratoma composed of mesenchymal, epithelial, and neural embryonal tissues. No other germ cell tumor components, such as embryonal carcinoma, yolk sac tumor, choriocarcinoma, or seminoma could be identified histologically. Clinical staging revealed no other tumor manifestations; in particular, both testes were normal and no retroperitoneal or mediastinal tumors were identified. Serum alpha-fetoprotein (AFP) and beta-human chorionic gonadotrophin (HCG) levels were normal. On the basis of the original histological classification of the tumor as a sarcoma an upper thigh amputation was performed. The amputation specimen showed an encapsulated tumor measuring 15 × 8 × 8 cm. The tumor was attached to the periosteum, but did not invade the bone. Its histology was similar to that of the biopsy. Three months later a retroperitoneal tumor became manifest and was resected. The
specimen measured $6 \times 4.5 \times 4.5$ cm and was composed of the same components as before. After another year an enlarged inguinal lymph node and retroperitoneal masses were noticed, and a fine-needle aspiration confirmed the recurrence of immature teratoma. The serum level of AFP was now slightly raised, HCG was in the normal range. Chemotherapy using carboplatin, etoposide, and bleomycin caused regression of the tumor and normalization of the serum AFP level. Further surgery was deemed unfeasible. The patient died 3 months later, with extensive retroperitoneal tumor masses.

**Case 2**

The patient was a 39-year-old white male with a disseminated extragonadal GCT histologically classified as immature teratoma (Oosterhuis et al., 1985). The localization of the primary tumor in the posterior mediastinum was established beyond doubt at autopsy. Twelve months elapsed between the patient's presentation with a subcutaneous metastasis of the trunk and his death due to local and metastatic tumor growth. The first chemotherapy, consisting of a combination of cisplatinum, vinblastine, and bleomycin (PVB), resulted in a partial remission that lasted 5 months. Residual metastatic tumor in the lungs was surgically removed. A relapse of the disease was treated with salvage chemotherapy using VP16-213 and cyclophosphamide, followed by autologous bone marrow transplantation, which again resulted in a partial remission of 5 months.

Following PVB, the residual tumor tissue consisted exclusively of mature teratoma. Apart from that, all the tumor tissue, either surgically removed or found at autopsy, was histologically predominantly composed of immature teratoma with small foci of mature somatic tissue. Serum AFP levels were slightly elevated in the last 2 months. Serum levels of HCG, lactate dehydrogenase (LDH), and CEA were consistently in the normal range. Minute amounts of AFP were immunohistochemically demonstrated in the immature teratoma component of the tumor.

**MATERIAL AND METHODS**

**Cytogenetic Analysis**

In case 1, cytogenetic analysis was carried out on two tumors (from the left thigh and the retroperitoneum) after short-term culture (5–7 days) as described previously (Castedo et al., 1989).

The karyotype of the tumor of case 2 has been published before (Oosterhuis et al., 1985). By using another banding method and elongated chromosomes, a better morphology of the chromosomes was achieved, which allowed us to refine the earlier karyotype description.

**In Situ Hybridization**

Bicolor double FISH experiments were carried out basically as described before (Suijkerbuijk et al., 1992, 1993; Olde Weghuis et al., 1994). In short, DNAs from the chromosome 6, 7, 11, and 12 specific plasmid libraries (i.e., pBS-6, -7, -11, and -12: Collins et al., 1991) were labeled with biotin-14-dATP (Life Technologies, Breda) or digoxigenin-11-dUTP (Boehringer Mannheim, Germany) following standard nick-translation. The labeled DNAs of pBS-6 and -11, and pBS-7 and -12, respectively, were coprecipitated with $15 \times$ Cot-1 DNA, preannealed for 15 minutes at 37°C and, subsequently, used as a hybridization mix on metaphase of tumor cells. Chromosomes and chromosomal segments hybridizing to biotin- or digoxigenin-labeled probes were visualized using a layer of fluorescein isothiocyanate (FITC)-conjugated avidin (Vector Laboratories, Burlingame, CA) and alternating layers of rabbit anti-FITC and FITC conjugated mouse anti-rabbit (Jackson ImmunoResearch, West Grove, PA) or Rhodamin-conjugated sheep anti-digoxigenin antibodies (Boehringer Mannheim) and Texas Red-conjugated donkey anti-sheep antibodies (Jackson ImmunoResearch), respectively. Counterstaining of the chromosomes was performed with the blue DNA-specific dye DAPI (Sigma, St. Louis, MO). Chromosomes were studied under a Zeiss Axiophot epifluorescence microscope, equipped with appropriate filters for the visualization of FITC, Rhodamin/Texas Red and DAPI fluorescence, as well as the simultaneous visualization of FITC and Texas Red fluorescence (Omega double filter; Omega Optical, Inc., Brattleboro, VT). Acquisition of separate digital images (for Texas Red, FITC, and DAPI) was established using a Photometrics high-performance CH250/A cooled CCD camera (Photometrics, Tucson, AZ) interfaced onto a Macintosh Quadra 900 computer. The images were superimposed and displayed on red-green-blue pseudocolors on the computer screen by means of the BDS-Image™ FISH software package (Biological Detection Systems, Inc., Rockville, MD). Photographs were made from the computer screen on Kodak EPP 100 Plus colorslide film using a Polaroid Quickprint.
RESULTS

Karyotyping

In both tumors of case 1 (left thigh and retroperitoneum), a total number of 10 cells was analyzed. All analyzed metaphases from case 1 showed the same karyotype with the description:

46,XY,der(6)t(6;11)(p21;q13)t(6;6)(q23;p22),
der(7)t(6;7)(p21;p22)t(6;6)(p22;q23),del(11)(q13).

Because of improved banding and high resolution techniques we could refine the karyotype description of the earlier published extragonadal GCT with abnormal chromosomes 6 and 11 (case 2) (3). All metaphases analyzed showed the same karyotype with the revised description:

46,XY,der(6)t(6;6)(q21;q16),der(6)t(6;6)(p22;q23)
t(6;11)(q16;q13),der(11)t(6;11)(p21;q13).

The two tumors have the following common chromosomal breakpoints: 6p21, 6p22, 6q23, and 11q13. The same chromosome regions, 6p22::6q23 and 6p21::11q13 are involved in fusions in both tumors (Fig. 1).

Peripheral blood from both patients showed a normal 46,XY karyotype.

FISH Analysis

Bicolor double FISH analysis was carried out on both tumors, using chromosome 7 and 12 or 6 and 11 specific paints. In case 2, chromosomes 7 and 12 appeared to be normal, as expected. Positive signals for chromosomes 6 and 11 were present according to the der(6)t(6;6), the der(6)t(6;6)t(6;11), the der(11)t(6;11), and one normal copy of chromosome 11. In case 1, two normal copies of chromosome 12 were present. Positive signals for chromosomes 6 and 11 were present according to one normal copy of chromosome 6, the der(6), the der(7), the del(11) of the t(6;7;11), and one normal copy of chromosome 11. In Figure 2, the normal chromosomes 7 and 12 of case 2 (Fig. 2A) and the t(6;7;11) of case 1 (Fig. 2B) are shown.

DISCUSSION

Cytogenetics

The published, complete karyotypes of seven malignant extragonadal germ cell tumors (Albrecht et al., 1993; Chaganti et al., 1989; Dal Cin et al., 1989; De Bruin et al., 1994; Mann et al., 1983; Oosterhuis et al., 1991; Shen et al., 1990) in addition to the present case and the revised karyotype of our previously reported case (Oosterhuis et al., 1985), are listed in Table 1. The karyotypes of four more mediastinal GCTs have been reported: two by Samaniego et al. (1990), and two by Rodriguez et al. (1992). Because of the limited available clinical data, these cases were not included in the table. However, they are considered in the discussion.

Cases 1 and 2 are pseudo-diploid and show complex chromosomal translocations, with common breakpoints in chromosomes 6 and 11. They lack isochromosome 12p, or other rearrangements involving 12p, as convincingly demonstrated by FISH.

Cases 3 through 9 lack the specific translocations of cases 1 and 2, and may have an isochrome 12p, the specific chromosomal abnormality frequently found in gonadal GCTs (Atkin and Baker, 1982; De Jong et al., 1990). It is present in cases 3, 4, and 7, and probably also in case 5 (Dal Cin et al., 1989). The tumors lacking i(12p) may have amplification of 12p in other chromosomal rearrangements similar to the pattern found in “i(12p)-negative” germ cell tumors of the adult testis. These tumors have extra copies of 12p hidden in various marker chromosomes, as demonstrated by painting of metaphase cells (Suijkerbuijk et al., 1992). The tumors of this second category may be (near)diploid or polyploid and may have non-random numerical abnormalities (e.g., +7, +21, and −13) similar to those found in testicular GCTs (De Jong et al., 1990). In testicular GCTs, the breakpoints of cases 1 and 2 are rare, and the fusions 6p22::6q23 and 6p21::11q13 have not been demonstrated in our material of 95 cases (unpublished data).

Cytogenetic Classification

The available karyotypic data on malignant extragonadal GCTs reviewed above allow the following cytogenetic classification:

Type 1. Diploid tumors with specific structural chromosomal abnormalities of chromosomes 6 and 11 (cases 1 and 2).

Type 2. Near-diploid or polyploid tumors which may have i(12p), and multiple non-random numerical abnormalities, similar to the chromosomal abnormalities found in testicular germ cell tumors (cases 3 through 9).

The four mediastinal GCTs reported by Samaniego et al. (1990) and Rodriguez et al. (1992)
Figure 1. Karyotype and partial karyotype and schematic representation of the structurally abnormal chromosomes of case 1 (A) and case 2 (B). Both tumors show common breakpoints: 6p21, 6p22, 6q23, and 11q13, with the same chromosomal regions, 6p22:6q23 and 6p21:11q13, involved in fusions.
are all type 2 tumors. All four have one or more copies of i(12p), three are polyploid.

Clinical-Pathological Correlation

The two tumors of type 1 occurred in male patients who were old as compared to the four patients with type 2 extragonadal GCTs of the anterior mediastinum. Both presented with bloodborne metastasis to the soft tissues (lower extremity and trunk). The primary tumors were localized in the retroperitoneum (case 1) and in the posterior mediastinum (case 2). Both patients developed slight elevation of AFP after multiple recurrences. Other serum tumor markers were negative. Not-

Figure 2. FISH analysis of tumors of case 2 (A) and case 1 (B) using chromosome 7 (yellow-green) and 12 (red) or 6 (yellow-green) and 11 (red)-specific paints. The differently labeled normal and translocation-derived chromosomes are marked. Chromosomes 7 and 12 appear to be normal in A (case 2), as expected (see text), whereas a clear t(6;7;11) is visualized in B (case 1).
<table>
<thead>
<tr>
<th>Case</th>
<th>Age/sex</th>
<th>Anatomical site</th>
<th>Serum markers</th>
<th>Histology</th>
<th>Karyotype</th>
<th>Follow-up</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>Type 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>1</td>
<td>65/M</td>
<td>Retroperitoneal</td>
<td>AFP ↑ HCG normal</td>
<td>TI</td>
<td>46,XY,der(6)(q;6)(p12;13)(q12;6)(q23;p22), der(7)(q;6)(p21;p22)(q;6)(p22;q23),del(11)(q13)</td>
<td>Died after 21 months of metastatic disease</td>
<td>This report</td>
</tr>
<tr>
<td>2</td>
<td>39/M</td>
<td>Posterior mediastinal</td>
<td>AFP ↑ HCG normal</td>
<td>TI</td>
<td>46,XY,der(6)(q;6)(q16),(q21;q16),der(6)(p21;q23),der(11)(q;6)(p21;q13)</td>
<td>Died after 12 months of local and metastatic disease</td>
<td>Oosterhuis et al. (1985)</td>
</tr>
<tr>
<td><strong>Type 2</strong></td>
<td></td>
<td></td>
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<tr>
<td>3</td>
<td>18/M</td>
<td>Anterior mediastinal</td>
<td>AFP ↑ HCG ↑</td>
<td>SE EC TD YO</td>
<td>47,XY,-13,+21,+i(12p)</td>
<td>Died after 13 months of recurrence</td>
<td>Dal Cin et al. (1989)</td>
</tr>
<tr>
<td>4</td>
<td>19/M</td>
<td>Anterior mediastinal</td>
<td>AFP ↑ HCG ↑</td>
<td>TI TD YO</td>
<td>48–49,XY,+1,+6,+i(12p)</td>
<td>Died after 12 months of secondary ANLL</td>
<td>Chaganty et al. (1989)</td>
</tr>
<tr>
<td>5</td>
<td>18/M</td>
<td>Anterior mediastinal</td>
<td>AFP ↑ HCG ↑</td>
<td>EC</td>
<td>50,XX,Y,+7,+21,+mar(l2q11)</td>
<td>Died after 10 months of local and metastatic disease</td>
<td>Mann et al. (1983)</td>
</tr>
<tr>
<td>6</td>
<td>26/M</td>
<td>Anterior mediastinal</td>
<td>AFP ↑ HCG ↑</td>
<td>TI TD</td>
<td>76,XY,+,Y,+Y,+1,+2,+3,+3,+5,+5,+6,+6,+7,+7,+8,+8,+10,+11,+12,+13,+15,+16,+17,+17,+19,+19,+20,+21,+21,+22,+del(9)(q21q22)</td>
<td>Died after 2 years of secondary ANLL</td>
<td>Oosterhuis et al. (1991)</td>
</tr>
<tr>
<td>7</td>
<td>16/M</td>
<td>Midline brain pineal</td>
<td>AFP ↑ HCG ↑</td>
<td>EC CH</td>
<td>64,XY,+X,+3,+7,+7,+8,+8,+12,+14,+20,+21,+21,+22,+del(1),+der(1),+del(2),+i(12p),+i(12p),+del(17),+del(20),+del(22),+mar</td>
<td>Alive after 22 months follow up</td>
<td>De Bruin et al. (1994)</td>
</tr>
<tr>
<td>8</td>
<td>14/M</td>
<td>Midline brain pineal</td>
<td>AFP ↑ HCG ↑</td>
<td>SE EC TD</td>
<td>78,X,-Y,+X,+X,+2,+2,+3,+3,+4,+5,+6,+6,+7,+7,+8,+9,+10,+12,+12,+14,+15,+16,+17,+19,+20,+20,+21,+21,+21,+21,+22,+i(12q11),+c(12q),+der(1)X(1)(p11),+der(1)X(1)(q12),+der(1)X(1)(q12),+der(1)X(1)(p11),+der(1)X(1)(q11),+der(1)X(1)(p11),+der(1)X(1)(q12),+der(1)X(1)(q12),+der(1)X(1)(p11),+der(1)X(1)(q11),+der(1)X(1)(p11),+der(1)X(1)(q12),+der(1)X(1)(q12),</td>
<td>No follow-up available</td>
<td>Shen et al. (1990)</td>
</tr>
<tr>
<td>9</td>
<td>11/M</td>
<td>Midline brain pineal</td>
<td>Not reported</td>
<td>SE</td>
<td>81,XY,+X,+Y,+1,+2,+2,+add(3) (p21)+5,+5,+6,+6,+6,+7,+8,+8,+8,+8,+add(9)(p13)+10,+add(12)(p11)+14,+14,+15,+15,+16,+17,+17(q10),+18,+18,+21,+21,+22,+HSR,+(?)+5mar</td>
<td>No follow-up available</td>
<td>Albrecht et al. (1993)</td>
</tr>
</tbody>
</table>

*AFP = alpha fetoprotein; HCG = human chorionic gonadotropin; CH = choriocarcinoma; EC = embryonal carcinoma; TD = teratoma differentiated; TI = teratoma immature; SE = seminoma; YO = yolk sac tumor; ANLL = acute nonlymphocytic leukemia.

Although extensive locoregional and systemic treatment, both patients died of their tumors. Primary tumors and untreated metastases of both patients were composed of immature teratoma. Minute amounts of AFP could be demonstrated in the tumor tissue of the second patient shortly before death (Oosterhuis et al., 1985). Four of the seven listed tumors of type 2 occurred in young adults, and were localized in the anterior mediastinum. The tumors in the three youngest patients were localized in the midline of the brain. The patients presented with complaints from the primary tumors, without manifest metastatic disease. The serum tumor markers, most of-
ten AFP and HCG, were elevated at presentation. Six tumors were histologically classified as nonseminomatous GCTs with the same mixed histology which is encountered in testicular GCTs of adults, including the presence of a seminoma/germinoma component in some tumors (cases 3 and 8). Case 9 was histologically classified as a germinoma. One of the patients with GCTs of the midline of the brain is alive without evidence of disease after a combination of radiotherapy and chemotherapy (follow-up 21 months). The four patients with mediastinal tumors died, notwithstanding extensive surgery and chemotherapy, two of them with secondary leukemias, an established risk of extragonadal GCTs of the anterior mediastinum (Chaganti et al., 1989).

**Histogenesis**

The remarkable resemblance in terms of histological composition and chromosomal constitution, between testicular GCTs of adults (and certain ovarian GCTs), and the extragonadal GCTs of type 2, suggests that they have a similar histogenesis, and similar cells of origin.

It is now generally accepted that the common precursor of all GCTs of the adult testis with the exception of spermatocytic seminoma, is carcinoma in situ (Skakkebaek et al., 1987), composed of tumor cells which are the neoplastic counterparts of primordial germ cells. Type 2 extragonadal GCTs are probably also derived from primordial germ cells, which have migrated, by an as yet unclarified mechanism, to the anterior mediastinum/thymus, and the midline of the brain. One could argue that the presence of i(12p), in type 2 extragonadal GCTs, is the best evidence yet that primordial germ cells do migrate to the midline of the brain and the thymus. The fact that they survive in these anatomical localizations through childhood and adult life suggests that they are more than embryonal vestiges, but have an as yet obscure functional meaning (Friedman, 1987).

It is possible that type 1 tumors are also derived from germ cells. Immature teratomas of the ovary which have a very similar histology and may behave as frankly malignant tumors, are derived from germ cells. Ovarian immature teratomas, however, are karyotypically different from type 1 extragonadal GCTs (Surii et al., 1990). Alternatively type 1 tumors are derived from pluripotent embryonal cells, analogous to what is considered for sacral teratomas and teratomas of the head and neck of infants (Gonzales-Crussi, 1982). Too few of these tumors have been karyotyped to allow a comparison with type 1 extragonadal GCTs (Oosterhuis et al., 1990).

Animal models of teratoma/teratocarcinoma and yolk sac tumor in the mouse and the rat could support either hypothesis on the cellular origin of type 1 tumors. Testicular teratomas in 129 mice (Stevens, 1973) and ovarian teratomas of the LT mouse strain (Stevens and Varnum, 1974) are derived from germ cells. However, tumors with almost identical histology produced in mice by embryo transplantation (Damjanov and Solter, 1974) and in rats by fetectomy (Sobis et al., 1982), are derived from pluripotent embryonal cells.

**Diagnosis of the New Entity**

Suspicion of a case of type 1 extragonadal GCT should be raised by the clinical presentation, in particular the relatively high age of the patient, and by the diploid character of the lesion. The only way to prove a case as yet is by karyotyping. Lack of involvement of chromosome 12 in interphase cytogenetics (in situ hybridization for the centromere of 12; Looijenga et al., 1990) argues against a type 2 and for a type 1 tumor. In the future it may be possible to demonstrate the specific chromosomal fusion regions with molecular techniques (Sinko et al., 1994).

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