Isochromosome 12p-positive Pineal Germ Cell Tumor

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ABSTRACT

We report the chromosomal characteristics of a recurrent pineal non-seminomatous germ cell tumor in a 16-year-old male patient. This non-seminomatous tumor had the following components: embryonal carcinoma, teratoma, yolk sac tumor, and trophoblastic giant cells. Chromosome analysis showed a near-triploid karyotype (64 chromosomes), including two copies of an isochromosome 12p. This latter finding could be confirmed using 12p-specific competitive in situ hybridization techniques applied to cultured cells (T2219-P6 cell line) derived from the tumor. The present findings are in keeping with the hypothesis that isochromosome 12p formation is associated with the development of malignant extragonadal germ cell tumors.

INTRODUCTION

GCTs may arise from gonadal or extragonadal tissues and may contain one or more of the following histological components: germinoma (seminoma, dysgerminoma), teratoma (mature or immature), embryonal carcinoma, endodermal sinus tumor, and choriocarcinoma (1). Testicular GCTs in adults are characterized by a specific chromosomal abnormality, the i(12p) (for reviews, see Refs. 2–4). The i(12p) has also been demonstrated in a subgroup of ovarian GCTs (for a review, see Ref. 5), and in extragonadal GCTs (5–9). However, published karyotypes from two pineal gland GCTs (10, 11) both lacked an i(12p). In this report we describe the chromosomal characteristics of a pineal nonseminomatous GCT from a 16-year-old male patient, in whom two copies of i(12p)2 could be demonstrated. Therefore, this represents the first report on a malignant GCT of the midline of the brain with an i(12p).

CASE REPORT

A 16-year-old male patient was seen at the Utrecht Academic Hospital with signs of increased intracranial pressure. Limited upward gaze (Parinaud’s syndrome) and divergent strabismus of the left eye were also present. Computed tomographic scanning revealed hydrocephalus due to a large obstructing tumor in the pineal region, extending into the right temporal lobe. A ventriculoperitoneal drain was placed, followed by transcranial tumor extirpation 13 days later. Histological examination showed a nonseminomatous GCT with the following components: embryonal carcinoma, teratoma, yolk sac tumor, and trophoblastic giant cells (Fig. 1). Computed tomographic scanning and echography revealed no tumor localizations in testes, abdomen, or mediastinum. HCG and AFP were measured before surgery: the plasma concentration of HCG was 629 units/liter (normal, <5 units/liter), and the concentration in cerebrospinal fluid was 2190 units/liter. The plasma concentration of AFP was increased again concomitantly with tumor growth in the right cerebral hemisphere. Chemotherapy was instituted, consisting of 150 mg etoposide i.v., 1500 mg ifosfamide i.v., and 25 mg platinol i.v. in combination with mesna. The patient received 6 cycles of chemotherapy at intervals of 4 weeks; mild myelodepression was observed. This regimen resulted in a decrease in tumor size and a normalization of HCG and AFP values. Chemotherapy was complicated by grand mal seizures, 14 days after the first cycle, which were successfully treated with 150 mg phenytoin twice/day. The patient is presently, 26 months after diagnosis, at home receiving hormone substitution for panhypopituitarism and oral phenytoin treatment.

Cytogenetic Studies. A fresh tumor biopsy was finely minced with scissors and treated overnight with 1% (w/v) collagenase in RPMI 1640 medium containing 15% fetal calf serum, L-glutamine, and antibiotics. The following day the resulting cell suspension was centrifuged, resuspended in fresh medium, and plated into 25-cm² flasks. Cell growth was slow, and adequate chromosome preparations were obtained after 9 weeks of culture. Chromosome preparations were made by adding 0.2 μM/mL demecolcine (Sigma, St. Louis, MO) for 30 min, followed by routine harvesting procedures using 0.075 mol KCl and methanol-acetic acid fixation. Metaphases were stained with Atebrin for Q-band analysis, followed by C-bandning. The karyotypes were described according to the International System for Cytogenetic Nomenclature 1991 (12).

Isochromosome 12p Detection by Double-Fluorescence in Situ Hybridization. Double-fluorescence in situ hybridization experiments on metaphase spreads of the cell line T2219-P6 were carried out using a combination of two different probes, p-α-12H8 (13), which is specific for the pericentric region of chromosome 12, and M28 hybrid cell DNA (14), which is specific for the short arm of chromosome 12 (15). In situ hybridization was carried out as described previously (16).

RESULTS

Karyotype Analysis and in situ Hybridization. Twenty-seven metaphases were analyzed. The chromosome number ranged from 59–64 with a mode of 64. A stemline was present with the following karyotype: 64,XY,+X,del(1)(q12),+i(1)(q10),+del(2)(q13q24),+3,+7,+7,+8,+8,+12,+i(12)(p10),+i(12)(p10),+14,+der(17)t(Y;17)(q11;p11),+20,+del(20)(q12),+21,+21,add(22)(p13)+mar (Fig. 2).

Double-fluorescence in situ hybridization using M28 DNA (chromosome 12p specific) and p-α-12H8 (chromosome 12 centromere specific) on metaphase spreads of T2219-P6 showed the presence of three chromosomes with positively staining short arms and centromeres, representing three copies of chromosome 12 (Fig. 3). In addition, two i(12p) chromosomes, with both positively staining chromosomal arms (green) and centromeres (orange-red), were easily and unambiguously identified.

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4 The abbreviations used are: GCT, germ cell tumor; i(12p), isochromosome 12p; HCG, human chorionic gonadotrophin; AFP, a-fetoprotein; H & E, hematoxylin and eosin.

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Fig. 1. Nonseminomatous GCT of pineal gland with mixed histology. A. embryonal carcinoma of papillary type (H & E; color photograph magnification, × 150). B, teratoma and small foci of yolk sac tumor (H & E; photograph magnification, × 165). C, trophoblastic giant cells (H & E; color photograph magnification, × 165).

Fig. 2. Representative example of the chromosome pattern of the pineal nonseminomatous GCT. Arrows, characteristic i(12p).

**DISCUSSION**

To our knowledge, this is the first report that identifies the presence of isochromosomes for the short arm of chromosome 12 in a pineal GCT. The genuine character of the two i(12p) chromosomes has been confirmed by the double-fluorescence in situ hybridization technique (16) using probes specific for the short arm (M28 DNA) (14, 15) and the centromere (p-alpha-12H8) (13) of chromosome 12. Two cases reported so far (10, 11) showed polyploid karyotypes but lacked an i(12p).

The i(12p) occurs in about 80% of GCTs of the testis in adults, both in seminomas and in nonseminomatous GCTs (4). It has also been identified in a subgroup of ovarian GCTs and in GCTs of the anterior mediastinum (5–9). These GCTs have a similar histological composition. The most important characteristic is that they may be partially or entirely composed of seminoma or (dys)germinomas. These findings suggest that these GCTs may be derived from primordial germ cells or gonocytes (5, 17, 18). In view of the histological spectrum of GCTs found in the midline of the brain, one would expect to find the i(12p) in these tumors as well.

The present finding of i(12p) in a GCT of the pineal gland suggests that GCTs of this anatomical localization may be derived from primordial germ cells. Interestingly, the presence of primordial germ cells has not yet been directly demonstrated in the pineal gland. The i(12p) is the best available indirect evidence thus far that primordial germ cells can migrate to the pineal gland. In view of the consistent findings of the presence of i(12p) in GCT, amplification of 12p, due to i(12p) formation or otherwise (16, 19), may play an important role in the oncogenesis of this class of GCTs.
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Fig. 3. Double-fluorescence in situ hybridization on a metaphase spread of cell line T2219-P6 using both biotin-labeled M28 (chromosome 12p specific) and digoxigenin-labeled p-a-12H8 (chromosome 12 centromere specific) DNA as probes. Chromosomal regions hybridized with M28 or p-a-12H8 are indirectly visualized by fluorescein isothiocyanate or tetramethylrhodamine isothiocyanate, resulting in a green or orange-red fluorescent staining, respectively. Counterstaining of the chromosomes was performed with 4,6-diamino-2-phenylindole, yielding a blue fluorescence. Three copies of chromosome 12, identified by their positively staining short arms (arrows) and two copies of an isochromosome 12p (arrowheads) are easily identified by their different staining patterns (green/red/blue and green/red/green, respectively).
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