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Samuel Navarro · Rosa Noguera
Amando Peydró-Olaya · Antonio Llombart-Bosch

Bipolar (neural and myoblastic) phenotype in cell lines derived from human germ cell tumours of testis

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Abstract Non-seminomatous germ cell tumours of the testis (NSGCT) form a heterogeneous group of neoplasms. Cell lines derived from NSGCT may provide useful data concerning the biology of neoplasic precursor germ cells, differentiation of tumour stem cells and the relationship between various tissue components of these tumours. Four NSGCT were studied, two mixed tumours composed of teratocarcinoma, yolk sac and trophoblastic elements, and two malignant teratomas with a massive neuroectodermal component, equivalent to primary neuroectodermal tumours (PNET) of the testis. The explanted tumours gave rise to various cell populations, including epitheloid cells, flattened large cells, spindle cells and tear drop cells of neuroblastic type. Ultrastructurally, cultured cells expressed various degrees of neural and muscular differentiation: neurosecretory granules, intermediate filaments of glial nature, and filaments resembling Z-bands. Cultured cells showed the expression of several neural and muscular markers, including neurofilaments, cytokeratin, actin, desmin, neuron-specific enolase, glial fibrillary acidic protein and HNK-1. In addition, three cases expressed HBA-71 antigen and two expressed MyoD1 protein. All cases were aneuploid, and an isochromosome 12p, i(12p), was detected in three cases. Myoblastic and neural cells are the predominant tumour cells that grow in vitro, independent of the nature and composition of the primary germ cell tumour. A histogenetic relationship between germ cell tumours and small round cell tumours of childhood is suggested.

Key words Human germ cell tumour of testis · Cell culture · Neural differentiation · Myoblastic differentiation · Cytogenetic analysis

S. Navarro · R. Noguera · A. Peydró-Olaya
A. Llombart-Bosch (✉)
Department of Pathology, Medical School, University of Valencia, Avda. Blasco Ibañez 17, E-46010 Valencia, Spain

Introduction

Non-seminomatous germ cell tumours of the testis (NSGCT) form a heterogeneous group of neoplasms including embryonal carcinoma, yolk sac tumour, choriocarcinoma, teratoma and mixed tumours [34–56]; the latter comprise at least one-third of all testicular germ cell tumours (GCTs) [6, 24]. Teratomas are defined as germ cell tumours with elements derived from two or more embryonal layers; very occasionally they occur as pure testicular tumours [59]. Recently, a recognized variant of teratoma composed of neural cells and their precursors has been described as primitive neuroectodermal tumour (PNET) [1, 35, 36, 38].

The biological characteristics of germ cell tumours of the testis have been extensively studied, using in vivo and in vitro analysis [2–4, 7–9, 31, 42, 43, 52]. The existence of murine teratoma models and the establishment of several teratocarcinoma cell lines have provided useful data on the biology of the neoplastic precursor germ cell and on the relationship between seminoma and non-seminomatous tumours [7, 9]. In vivo and in vitro studies performed on teratocarcinoma cell lines have demonstrated a clear pluripotential capacity for differentiation in these cells; the expression of neural tissue is a common finding [8, 9, 49, 62]. Nevertheless, in vitro established cell lines usually maintain the character of a multipotent stem cell precursor, regardless of the tissue from which the explant was obtained. Several of these cell lines have been established for decades without losing their pluripotential nature [2, 9].

Association of neural and muscle cell expression has been reported since retinoid acid induced differentiation in a human germ cell line derived tumour (NCCIT). In the report on this work, association of neural marker expression as well as desmin positivity was observed within the same cells following xenografting in nude mice. No particular attention has been given to this finding, which was considered to be due to contamination with xenografted tissue from the animal [9].

Other studies, such as ploidy analysis, have demonstrated the high degree of aneuploidy that NSGCT ex-

Table 1 Clinical data (*AFP* alpha fetoprotein, *HCG* human chorionic gonadotrophin, *A* alive)

Case	Age	AFP	HCG	Metastases	Status
1	31	↑	↑	Lung, retroperitoneal lymph nodes	A, 11 months
2	23	↑	↑	No	A, 18 months
3	27	↑	↑	Paraortic lymph nodes	A, 9 months
4	21	↑	n	Lung, abdominal mesenteric tumour, retroperitoneal lymph nodes	A, 5 years

press [14, 37, 39, 40]. Conventional cytogenetics has shown that most germ cell tumours express the common chromosome marker isochromosome 12p [5, 11, 19, 46, 57, 60].

In this paper we present an in vitro study of cells derived from four NSGCT of the testis: two mixed germ cell tumours and two malignant teratomas with predominantly neuroectodermal tissue. The analysis shows the existence of a multiphenotype expression in which both neuroectodermal and muscle cell features were simultaneously present in every case. Three cases had the chromosomal marker isochromosome for 12p, i(12p), as described in NSGCT of the testis.

Materials and methods

Clinical data from the four cases studied are summarized in Table 1. The morphology and the state of differentiation were monitored with phase-contrast microscopy, electron microscopy, immunocytochemistry and the Western blotting technique. For immunocytochemical analysis, a panel of antibodies especially oriented to neural and muscular markers was used, as shown in Table 2. The expression of MyoD1 protein was assessed by the Western blotting technique. Both cytogenetic and ploidy analyses supported this study.

Tumor specimens were fixed in 10% formaldehyde and routinely processed. Paraffin sections from ten blocks were stained with HE and PAS. Glued paraffin sections were studied following the ABC peroxidase method as previously reported [22]. Antibodies, dilutions and sources are listed in Table 2. Small samples of fresh tumour tissue were fixed in 2.5% glutaraldehyde, postfixed in 1% osmic acid, dehydrated in graded alcohols and propylene oxide and embedded in epoxy resin. Ultrathin sections of selected areas were stained with uranyl-acetate and lead citrate and examined with an electron microscope (JEOL 100 B).

Table 2 Panel of antibodies used in this study

Antibody	Dilution	Source
Vimentin (cloneV9)	1/40	DAKO
Cytokeratin CAM 5.2	1/40	Becton-Dickinson
Neurofilament 68 kd	1/20	Boehringer
HNK-1	10 µg/ml	ATCC
Neuron-specific enolase	1/200	DAKO
Glial fibrillary acidic protein	1/300	DAKO
α-Fetoprotein	1/300	DAKO
Human chorionic gonadotrophin	1/300	DAKO
Actin (clon HHF-35)	1/1000	ENZO
Desmin (clon DE-R-11)	1/100	DAKO
HBA-71	1/20	SIGNET
MYOD1 Protein	1/50	NOVOCASTRA

Case 1

Grossly, the tumour was a grey irregular and multinodular mass 13 cm across, with abundant necrotic areas. Light microscopy of several sections revealed solid and papillary foci of embryonal carcinoma intermingled with loose areas reminiscent of endodermal sinus in appearance with Schiller-Duval bodies (Fig. 1a). Teratomatous elements were scarce and made up of immature blastema and isolated primitive neural tube structures. Ultrastructurally, tumour cells of the germinal type were arranged in solid nests, cytoplasmic organelles were scarce, and only cell–cell junctions, cilia and pseudolumina were prominent. Immunocytochemistry revealed reactivity for alpha fetoprotein (AFP) in embryonal areas and NSE and HNK-1 in the isolated areas with the neuroectodermal appearance.

Case 2

Macroscopic analysis showed a tumour mass of 10 cm, poorly delimited from the surrounding testis, with cystic and haemorrhagic areas. Microscopically, the tumour was composed mainly of embryonal carcinoma areas associated with yolk sac elements and with foci of giant and mononucleated trophoblastic cells. Teratomatous tissue was represented by immature blastema and isolated neural tube structures. Ultrastructurally, the fine appearance of tumour cells was similar to that described in case 1. Immunocytochemically, AFP and HCG were demonstrated in yolk sac and trophoblastic areas respectively, whereas NSE and HNK-1 were positive in the isolated zones of neuroectodermal appearance. Embryonal carcinoma areas were positive for cytokeratin and NSE.

Case 3

Grossly, the tumour measured 8.5 cm in diameter and had a grey appearance with cystic and haemorrhagic foci. Microscopic analysis revealed a heterogeneous tumour with teratomatous tissue composed mainly of neuroectodermal tubes, pseudorosettes and ependymal rosettes (Fig. 1b). Cartilage and epidermal structures were also seen. Germ cell areas of embryonal carcinoma were detected in association with teratomatous tissues. Ultrastructurally, embryonal carcinoma cells as described in cases 1 and 2 were observed with tubular areas of neuroepithelial aspect configurating polarized stratified cells with cilia, junctions and electron-dense granules. Immunocytochemically, neural markers such as NSE, HNK-1 and neurofilaments were detected in neural areas. Embryonal carcinoma foci were positive for CAM 5.2.

Case 4

Macroscopic study showed a tumour mass of 6 cm, which was solid and poorly delimited from testicular parenchyma. Histology revealed a tumour composed almost exclusively of neural structures:

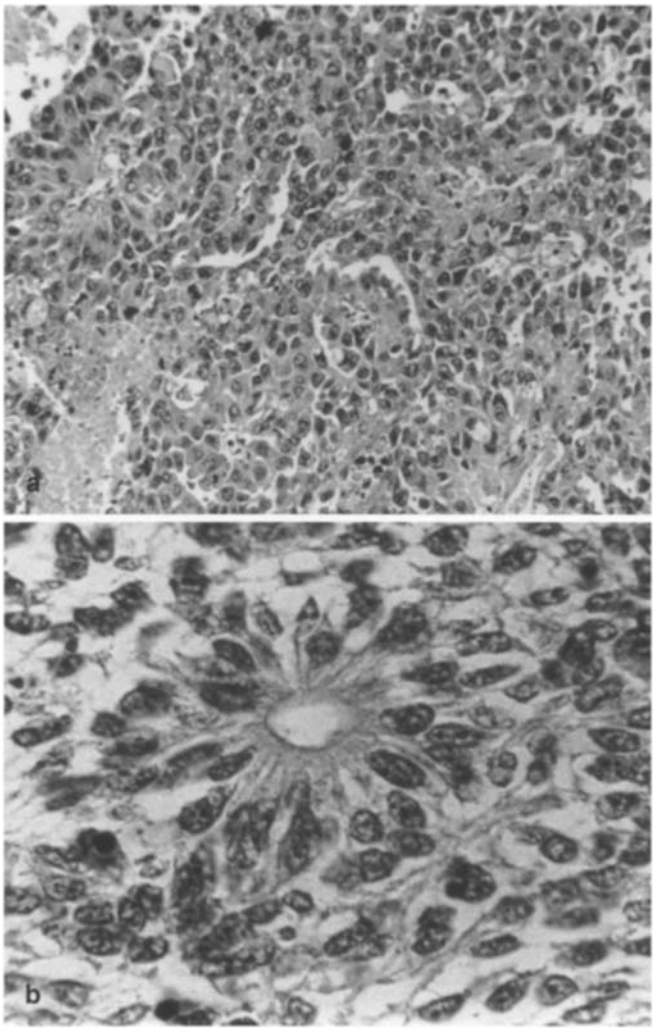


Fig. 1a, b Histological appearance of germ cell tumours of testis. **a** Case 1: solid and pseudopapillary areas of embryonal carcinoma. HE, ×40. **b** Case 3: foci of neural differentiation with a typical rossette. HE, ×65

neuroepithelial tubes of medulloepithelioma appearance, pseudorosettes of Homer-Wright and ependymal tubes and solid areas of glial nature. At electron microscopic level several neural features were seen: neural tubes with stratified neuroepithelium, cilia and junctions, neuroblastic rosette-like structures with neurosecretory granules and intermediate filaments. Immunocytochemistry revealed reactivity with antibodies to NSE, HNK-1, NF and GFAP. Malignant cells with myogenic phenotype were not seen in any primary tumour.

For tissue culture and cytogenetic analysis biopsy specimens were placed in serum-free RPMI 1640 medium at 4°C immediately after removal from the patient, and transported to the laboratory as soon as possible. The specimens were dissected with crossed scalpels and the fragments washed twice in phosphate-buffered saline (PBS) supplemented with antibiotics (penicillin 100 IU/ml plus streptomycin 100 mg/ml). Thereafter 2 mg/ml of collagenase IV (Cooper Biomedical, New York) and 100 mg/ml of DNAse (Sigma, St Louis, Mo.) were added to the tumour pieces and the medium for 30–60 min at 37°C, depending upon the amount of fibrous tissue present in the surgical material. Every 5–10 min the solution was vortexed briefly to facilitate the detachment of tumour cells from the stroma. The turbid supernatant was collected and centrifuged at 200 g for 5 min and the pellet rinsed twice with RPMI 1640 medium supplemented with 20% fetal bovine serum plus 1% L-glutamine + 1% penicillin–streptomycin. Finally the pellet was collected and plated on a 25-cm culture flask with the medium, which was changed every 3 days. Long-term cultures were examined for growth, and medium was changed twice a week. These cultures were tested for the presence of fungi, mycoplasma and/or bacteria and were free of any detectable contamination while used in the studies.

The cultures were maintained for 10 passages showing stability of the phenotypes and were progressively frozen and stored in liquid nitrogen. Cells for chromosome analysis were harvested on tumour tissue by the direct method (24 h). Short-term cultures (2–6 days) and cells from long-term cultures (2–6 months) were also used for the cytogenetic analysis.

Preparations were processed after 1 h with colcemid (0.1 mg/ml final concentration), swollen in 0.075 mol/potassium chloride, fixed twice in methanol acetic acid (3:1), dropped on cold, wet slides and aged 24 h in a 60°C oven. The Giemsa technique was used for staining and banding [23].

Electron microscopic analysis of cultures was carried out on cells from several passages. A cell pellet of the cultures was prepared by gently trypsinizing the monolayer, centrifuging at 800 rpm, washing once with PBS and twice with Sorensen's buffer, and then fixing the pellet in phosphate-buffered glutaraldehyde. The pellet was embedded in resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope (JEOL 100B).

The ABC peroxidase method was performed on cultured cells on chamber slides previously fixed with methanol-acetone at –20°C. For immunostaining, the antibodies listed in Table 2 were used. Western blotting for MyoD-1 protein was performed according to the Laemly and Towbin method [26, 53]. Total protein extracts were processed electrophoretically and transferred to nitrocellulose. ABC peroxidase method was performed and the peroxidase revealed with 4-chloro-1-naphtol.

Flow cytometric analysis was performed on representative fresh tissue samples, paraffin-embedded tissues and cell cultures. Cell suspensions from primary tumours were prepared by the method recommended by Schutte et al. [47]. The cells were stained with propidium iodide following the technique of Vindelov et al. [58]. Measurements were performed using an Epics Profile Analyzer and analysed with Multicycle Software (Phoenix Flow Systems, San Diego, Calif.). DNA content was expressed as a DNA index (DI) i.e., the ratio between tumour cells and diploid G1 cells. The FCM histograms were analysed with reference to the DNA index of distinguishable stemlines.

Results

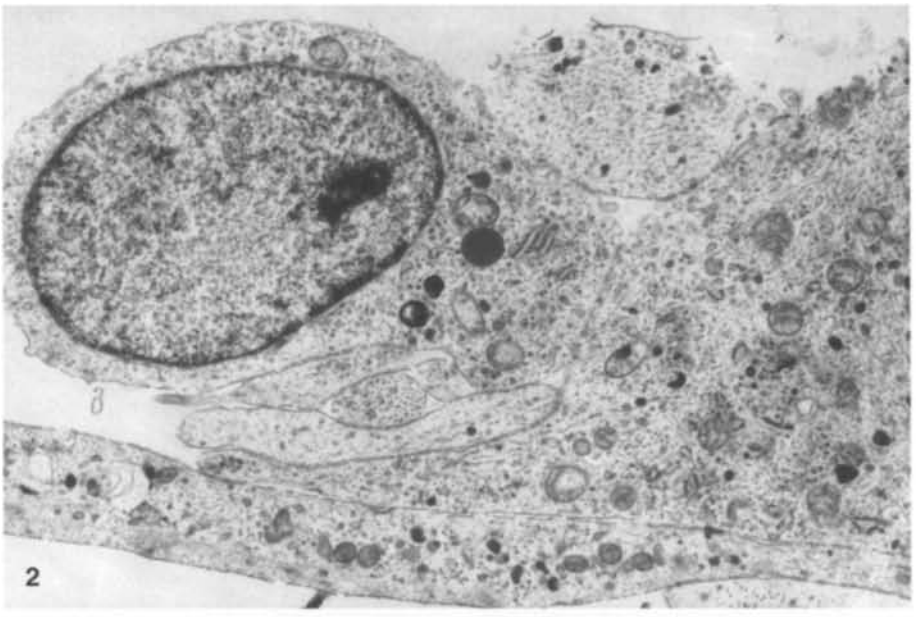
In phase contrast morphology, all cases showed similar features: multiple cell populations with epitheloid cells forming colonies, large flattened cells attached to substrate, spindle cells, and in cases 2, 3 and 4, small cells with cytoplasmic processes (tear-drop cells).

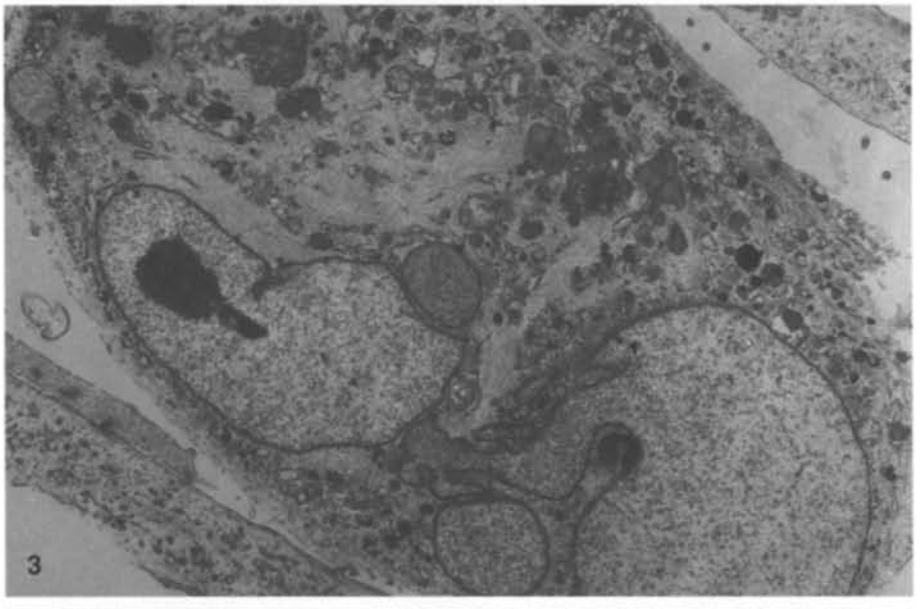
Ultrastructural study of cultured cells revealed cells of spindle-shaped appearance with oval nuclei and well-developed cytoplasm rich in intermediate filaments containing electron-dense granules of the neurosecretory type. In cases 2 and 3, these cells were detected in addition to large cells with round to oval nuclei and cytoplasm rich in thin and thick filaments configurating Z-band material. In case 4, in addition to the neuroblastic and myoblastic cells, epitheloid cells with intermediate filaments arranged in a perinuclear fashion, giving the appearance of glial differentiation, were also detected. Morphological results confirmed that in all cases the

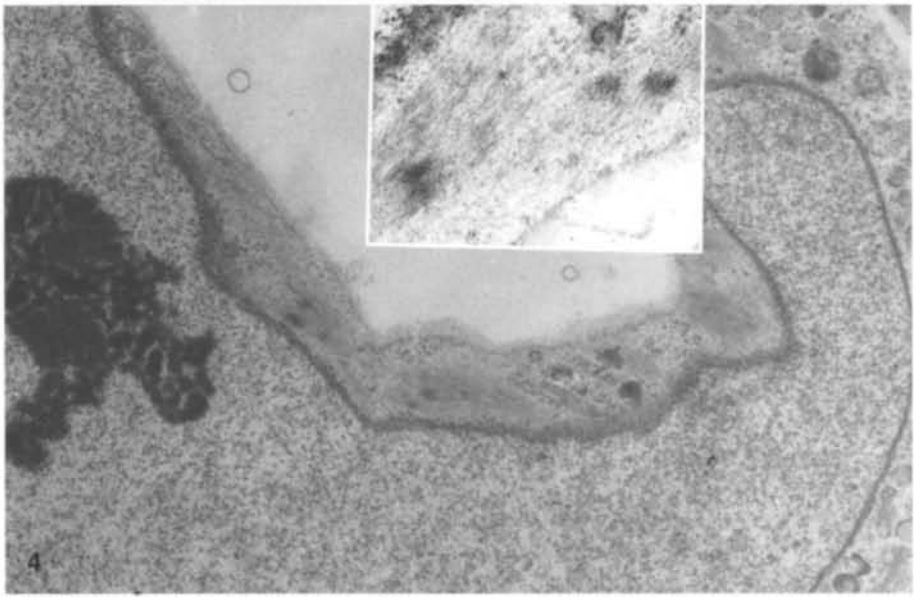
Fig. 2 Ultrastructural features of cultured cells from case 4, showing neuroblastic cell with several cytoplasmic processes filled with electron-dense granules. ×20,000

Fig. 3 Electron microscopy appearance of tumour cells from case 4 with cytoplasmic intermediate filaments arranged in a perinuclear fashion showing a glial appearance. ×18,000

Fig. 4 Ultrastructure of case 3, showing spindle-shaped cells with cytoplasmic bundles of thin and thick filaments configurating Z-bands. ×25,000. *Insert* Z-bands







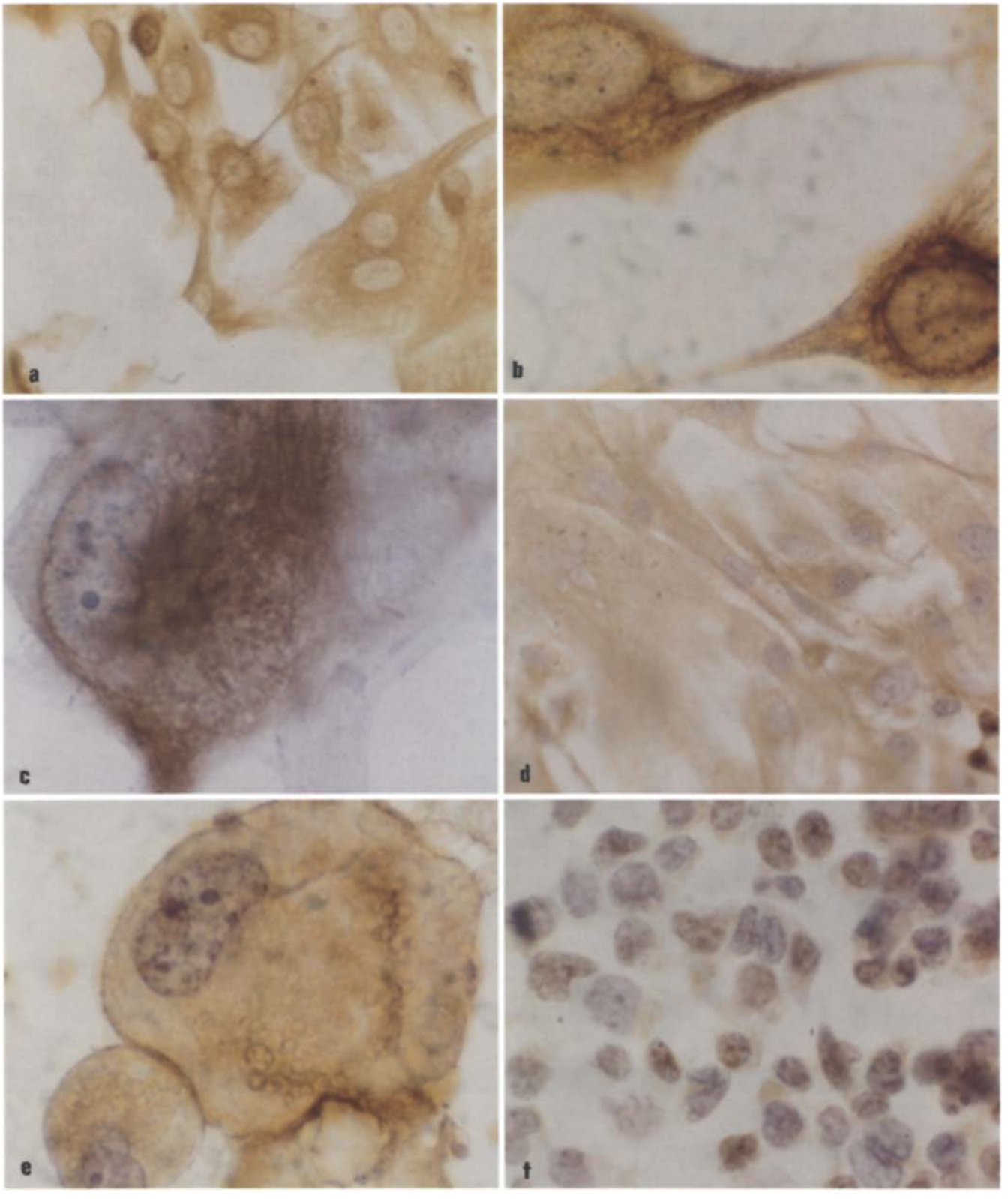


Fig. 5a–f Immunocytochemical results on cultured cells. **a** Cytoplasmic expression of cytokeratin filaments in case 1. ABC peroxidase, ×40. **b** Cytoplasmic expression of 68-kDa neurofilaments in case 3. ABC peroxidase, ×100. **c** Single cell showing filaments of desmin in case 4. ABC peroxidase, ×100. **d** Detection of actin filaments in case 1. ABC peroxidase, ×40. **e** Membranous and cytoplasmic detection of HBA-71 antigen in case 2. ABC peroxidase, ×100. **f** Nuclear staining of MyoD1 protein in case 4. ABC peroxidase, ×65

cells that were grown in culture medium had neural and muscular phenotypes (Figs. 2–4).

In case 1, the cells displayed reactivity against NSE, HNK-1, neurofilaments and cytokeratin (Fig. 5a). In cases 2, 3, and 4, the results against these antibodies were similar (Fig. 5b). Moreover, cells expressing such muscle markers as desmin and actin were also detected

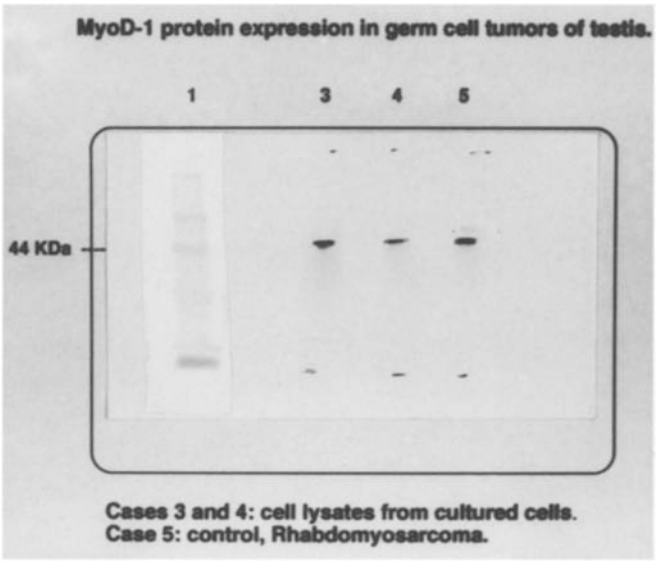


Fig. 6 Immunoblotting of cell lysates from cases 3 and 4. Detection of MyoD1 protein as a single band of 45 kDa

(Fig. 5c,d). In addition, GFAP-positive cells were seen only in case 4. In case 2, isolated large cells presented reactivity for AFP and HCG.

A finding of special interest was the expression of HBA-71 antigen observed in cultured cells from cases 2, 3 and 4 (Fig. 5e). No expression of this marker was seen in original tumours. HBA-71 has been described as a constant protein detected in the group of Ewing's sarcoma-PNET of bone and soft tissues. Another result that reinforced the muscular pathway of differentiation observed in our cultures was the detection of the myogenic regulatory protein MyoD1 in the cultures from cases 3 and 4 (Fig. 5f). This nuclear protein is expressed in various steps of muscle differentiation, and it is a marker typically expressed by rhabdomyosarcomas. In cases 3 and 4, typical nuclear staining was observed in tumour cells. Moreover, this expression was confirmed by immunoblotting which detected a single band of 45 kDa in the protein extracts of these cell cultures with antibody to MyoD1 (Fig. 6). MyoD1 protein expression was not observed in the original tumours. The results are summarized in Table 3.

Table 3 Global results of germ cell tumour study (TEM transmission electron microscopy, *IC* immunocytochemistry, *Ter* teratocarcinoma, *YST* yolk sac tumour, *Chorio choriocarcinoma,* PNET primitive neuroectodermal tumour, *EC* embryonal carcinoma cell, *dmin* double minutes, *HSR* homogeneous staining

Case	Diagnosis	TEM	IC	Cell culture morphology	Cell culture TEM	Cell culture IC	Ploidy	Cytogenetics
1	Mixed germ cell tumour (Ter +YST)	EC	αFP+NSE +HNK-1 +CAM 5.2	Epithelioid Spindle Flattened	Neural	NSE +HNK-1 +NF	1.37	dmin; HSR
2	Mixed germ cell tumour (Ter+YST +Chorio)	EC	αFP+HNK-1 +NSE+HCG +CAM 5.2	Epithelioid Spindle Flattened Tear-drop cell	Neural + muscular	NSE+actin +HNK-1 +NF+desmin +aFP+HCG HBA-71+	1.50	i(12p)
3	Teratocarcinoma + PNET	EC + Neural	NSE+HNK-1 +NF +CAM 5.2	Epithelioid Spindle Flattened Tear-drop cell	Neural + muscular	NSE+actin +HNK-1 +NF+desmin MyoD1+ HBA-71+	1.09	i(12p)
4	Teratocarcinoma + PNET	EC + Neural	VIM+NSE +HNK-1+NF +GFAP+CAM 5.2	Epithelioid Flattened Tear-drop cell	Neural +glial +muscular	NSE+GFAP +HNK-1+actin +NF+desmin HBA-71+ MyoD1+	1.14	i(12p)

Table 4 Cytogenetic analysis (*P* passage culture, *mar* markers)

Case	Culture	Cells counted/ analysed	Range	Mode	Karyotype
1	30 days/P-2	8/5	37–93	46 58	46 XY, dmin 58, HSR, +mar
2	15 days/P-2	20/5	61–68	68	68, XXY, dup(1)(q21→q42), −4, del(5)(p13), +8, −10.−11, +12, +i(12p), −22, r(22)(p1 1q13)
3	40 days/P-2	40/9	51–67	60	60 XX, +1, +2, +3, +6, +7, +12, +i(12p), +14, +17, +19, +t(19;1)(q13;q25), +20, +21, +21
4	7–27 days/P-1,3	37/3	15–68	64	64, +i(1q), +del(2)(p11), +i(12p), +del(14)(q24), +del(16)(q13), +del (20)(p11), +3 mar

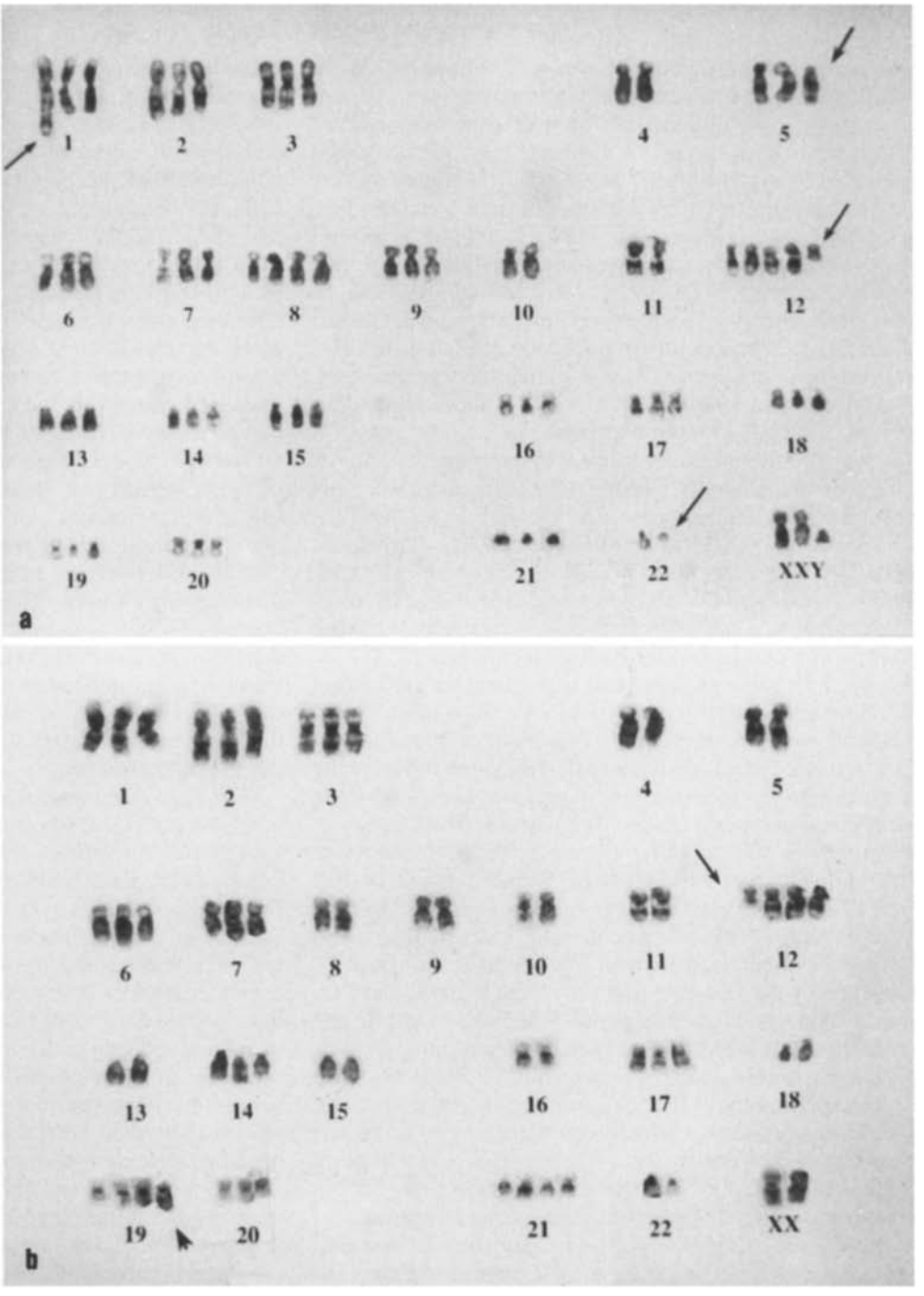


Fig. 7a, b Karyotypes of **a** case 2 and **b** case 3. Both cases show characteristic cytogenetic aberration in testicular germ cell tumours, isochromosome for 12p, i(12p)

Table 4 shows cytogenetic results. The modal chromosome number was hypotriploid (ranging from 58 in case 1 to 68 in case 2) with non-random acquired chromosome abnormalities. Case 1 showed two unrelated clonal karyotypes: 46,XY with double minutes (dmin) and 58,XY with unknown markers and homogeneous staining regions (HSR). Cytogenetics confirmed results already reported by other groups: isochromosome 12p appears to be a consistent genetic marker of germ cell tumours of the testis and was seen in three cases, cases 2, 3, 4 (Fig. 7). Only in case 1 were we unable to demonstrate this anomaly.

The DNA content of the four cases showed consistent aneuploidy. The DNA index of each case is shown in Table 3 with the rest of the results. These results confirm previous reports on germ cell tumours of the testis.

Discussion

NSGCT of the testis are a diverse group of tumours, which show a pluripotential capability to differentiate to embryonic and/or extraembryonic neoplastic tissues. In

all of these neoplasms (yolk sac tumour, embryonal carcinoma, choriocarcinoma, teratoma, and mixed tumours) stromal tissue is commonly found to be intermixed within the neoplasic germ cells. This stroma has an important role: in yolk sac tumours, the spindle-cell component of the myxomatous areas is composed of pluripotential cells that have the capacity to form differentiated mesenchymal tissues such as skeletal muscle and cartilage [31]. Moreover, in these tumours the cellular proliferation with its spindle cell component may give rise to distinctly "sarcomatoid patterns" [56]. In malignant teratomas, immaturity is often expressed as a cellular stroma distributed in a periepithelial fashion. Components displaying high-grade immaturity include neuroepithelium, blastematous Wilms'-like tissues and embryonic rhabdomyoblastic cells [55]. In our cases, the presence of stroma was prominent in the two mixed germ cell tumours, while in the other two neural tube structures were the predominant component.

Several human germ cell tumour cell lines have been established in vitro and studied in vitro and in xenografts. Various study methods were used including morphological, immunohistochemical, cytogenetic, electron microscopy and molecular biology techniques [2, 4, 7, 9, 42, 52, 62]. Most of these cell lines indicate pluripotent developmental capacity with foci of embryonal carcinoma, yolk sac tumour, trophoblastic giant cells and immature somatic tissues, indicating that they can differentiate into derivatives of extraembryonic lineages of all three embryonic germ cell layers. The cell lines lack neuroectodermal or muscle cell differentiation [52], and these two elements have seldom been found in the same cell line [9]. Neural markers and muscle cell determinants such as glial fibrillary acidic protein, neurofilaments and desmin have been detected in a pluripotent human germ cell tumour-derived cell line only after retinoic acid-induced differentiation. The NSGCT-derived stem cell line studied here, with multidirectional differentiation capacity, is comparable to the developmentally pluripotent embryonal carcinoma cell lines described by Andrews et al. [4]. However, some differentiation pathways could not be confirmed by electron microscopy or Western blotting in this line. Desmin-positive cells were found only in xenografted tumours, and Damjanov et al. [9] postulated that these cells might be of host origin, since no desmin-positive cells were found in vitro. That was not the case for neural markers (GFAP and neurofilaments), which were observed both in vitro after retinoid acid treatment and in xenograft solid tumours. Even though two of our cases presented massive neuroectodermal development in vivo, demonstrated by the immunocytochemical expression of several neural markers, coexpression of actin and desmin after in vitro studies was also observed. Cultured cells from two cases expressed MyoD1 protein, a muscle-regulatory protein detected in rhabdomyosarcomas [12, 13, 29].

Similar biphenotypic expression has been described in neuroblastoma cell lines [44, 51]. Morphologically, these cell lines have at least two populations: the N-type cells that exhibit neuronal phenotypic markers and the epithelial-like S-type cells (substrate adhesive cells) that resemble glial or mesenchymal cells. Moreover, muscle markers (actin and desmin) have been described in S-type cells [51]. It is known that ectomesenchyme (mesoectoderm) is a part of neural crest derivatives including neural cells of the peripheral nervous system, Schwann cells, perineural cells of the nerve sheaths, melanocytes and endocrine and paracrine cells. Muscle cells derived from this ectomesenchyme are part of the head and face as well of the vessel walls, mainly the walls of the large arteries derived from the branchial arch [27]. We hypothesize that the stromal pluripotential tissue of our cases has given rise to the neuroectodermal and muscular cells that predominate in the cultures. An ectomesenchymal nature should be ascribed to these cells.

Another model that could explain our findings is that of medulloblastoma. As is known, medulloblastoma belongs to the category of cPNET and therefore shares many similarities with other embryonal neoplasms [10, 21, 32, 45]. Several medulloblastoma and PNET cell lines have been used to gain insights into the characterization of the maturation of human neuroblastic malignancies. Trojanowski [54], using a number of these cell lines, hypothesized that cPNET partially recapitulates the different stages of normal human neuroblasts and that their neoplastic transformation is arrested at specific developmental stages.

TE671 is a cell line obtained from a cerebellar medulloblastoma [30, 33, 61]. It shares common biological and cytogenetic similarities with the RD rhabdomyosarcoma cell line [16, 29]. Based on cytogenetics, transfection of DNA into NIH 3T3 cells and DNA fingerprint analysis, Stratton et al. [50] concluded that both are derivatives of the same line; thus, the morphological and biological characteristics of this presumptive medulloblastoma cell line should be ascribed to rhabdomyosarcoma rather than to medulloblastoma. Simultaneous biphasic neural and muscular differentiation of TE671 and some cPNET in vivo shows that the biphasic differentiation of cells in teratomas studied here could occur under several conditions and several tumour types.

The existence of childhood sarcomas displaying multilineage differentiation is well known; among them the coexistence in the expression of primitive neural features and myogenic phenotype is the most common manifestation. These neoplasms, considered to be primitive sarcomas, possibly represent tumours derived from the ectomesenchyme. Moreover, a myogenic immunophenotype is known to be a feature of desmoplastic small round cell tumour of the abdominal cavity, retroperitoneal spaces and pleura [17, 18, 20, 41, 47]. This neoplasm shows not only desmin expression but also epithelial markers and vimentin together with a number of neuroectodermal differentiation epitopes. HBA71 is also clearly detectable, as occurs in Ewing's sarcoma-pPNET of children and young adults [15, 44]. Nevertheless, desmoplastic round cell tumours show a specific chromosomal abnormality close to, but differing from, that seen

in Ewing's sarcoma-pPNET: t(11;22) (p13;q11/q12). While the affected 22 region is close to, but not identical to, the locus involved in Ewing's sarcoma-pPNET, the p13 region is coincidental with the locus of the Wilms' tumour suppressor gene [25]. Thus, the multidirectional expression of desmoplastic round cell tumour is similar to that present in Wilms' tumours of the kidney. Nevertheless, none of these tumours has shown the presence of the most common and characteristic cytogenetic aberration in testicular germ cell tumours.

Cytogenetic studies have shown both increase and reduction of copy number of whole chromosomes or part of them in germ cell tumours. An isochromosome for the short arm of chromosome 12, i(12p), is the most common aberration observed [5, 19, 60]. Polyploidization and the characteristic chromosomal abnormality of GCT, i(12p), were found in three of our cases; both are important steps in the oncogenesis of GCT [11, 46, 57]. The functional importance of i(12p) remains unknown. Recently, it has been reported that high frequencies of loss of heterozygosity and allelic imbalance at several loci indicate that inactivation of several tumour suppressor genes may be of importance in developing testicular germ cell tumours [28].

We conclude that in our cultures a cell population derived from an ectomesenchymatous stroma is the predominant cell type that grows, showing neural and muscle phenotypes and retaining the specific chromosomal marker of germ cell tumours of the testis.

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