J. Appl. Genet. 41(2), 2000, pp. 123-131

Cryptic rearrangements of chromosome 12 in testicular germ cell tumors with or without the specific i(12p) marker

Beata GRYGALEWICZ, Barbara PIEŃKOWSKA-GRELA, Renata WORONIECKA

Cytogenetic Laboratory, The Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Warszawa, Poland

Abstract. Isochromosome of the short arm of chromosome 12 [i(12p)] is a highly specific chromosome abnormality of human testicular germ-cell tumors (TGCTs). It has been detected in 80% of cases. Other abnormalities that involved 12p-derived material have been observed in the remaining 20% of cases. In our study, cryptic rearrangements of the short arm of chromosome 12 were distinguished by fluorescence in situ hybridization (FISH) in eight cytogenetically abnormal TGCTs. This group included multiplicated material of 12p-arm in both i(12p)-positive and i(12p)-negative tumors. Such a common occurrence of chromosome 12 short arm rearrangements and overrepresentation of 12p-material confirms that yet unidentified gene(s) on 12p can play an important role in oncogenesis of TGCTs.

Key words: chromosome 12 aberrations, FISH, testicular germ cell tumor.

Introduction

Human testicular germ cell tumors (TGCT) can be divided (clinically and histologically) into two groups: seminoma (SE) and nonseminoma (NS) (MOSTOFI et al. 1987, MURTY, CHAGANTI 1998,). Both types of TGCTs develop from a common precursor, known as carcinoma in situ (SKAKKEBAEK et al. 1987) or intratubular germ cell neoplasia (OOSTERHUIS et al. 1989). Despite the hypertriploid DNA content for CIS and SE and hypotriploid DNA content for NS (OOSTERHUIS et al. 1989, EL-NAGGAR, et al. 1992, de GRAAFF et al. 1992), they show a striking similarity in chromosome constitution (de JONG et al. 1990),

Received: January 2000.
Correspondence: B. GRYGALEWICZ, Cytogenetic Laboratory, The Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, ul. K.W. Roentgen 5, 02-781 Warszawa, Poland.

depending on pathogenetic origin. The most common and highly indicative change is the formation of an isochromosome of a short (p) arm of chromosome 12-[i(12p)] (ATKIN, BAKER 1982). More than 80% of all TGCTs display one or more copies of such izochromosomes – [i(12p)] (RODRIGUEZ et al. 1992, GEURTS-VAN-KESSEL et al. 1993). In a relatively small group of these tumors without i(12p) other abnormalities involving chromosome 12 are usually found (GIBAS et al. 1986). Occurrence of 12p abnormalities is common in i(12p)-negative TGCTs. These abnormalities are analogous to i(12p) and may contribute to tumor development (SUIJKERBUIJK et al. 1993). Extra aberrations involving chromosome 12 can be found not only in i(12p)-negative but also in i(12p)-positive TGCT (SUIJKERBUIJK et al. 1992).

This study was carried out to identify cryptic rearrangements of chromosome 12 in primary testicular tumors. Chromosome-12-derived markers were identified using FISH and GTG re-examination in a selected group of tumors. All of these TGCTs appeared to contain 12p abnormalities, resulting in a distinct mode of overrepresentation. Our data concurs with the suggestion that the occurrence of 12p abnormalities is common in TGCT and that these abnormalities may contribute to tumor development.

Material and methods

Tumor material, cell culture and cytogenetic analysis

Fifteen tumor specimens (six seminomas and nine nonseminomas) were obtained from patients undergoing standard surgery for TGCT at the Maria Skłodowska-Curie Memorial Cancer Center. Histopathological diagnosis was performed by the Department of Pathology according to standard WHO criteria. Cells were cultured in a 5% CO_2 atmosphere at 37°C. The growth medium was composed of RPMI 1640, 15% fetal calf serum, 200 mM L-glutamine, 5 mg/ml insulin and antibiotics (penicillin 100 units/ml, streptomycin 100 mg/ml). Cells for cytogenetic analysis were harvested and proceeded following short-term culture according to conventional methods. Metaphase spreads were prepared according to standard procedures using methanol with acetic acid for fixation.

Chromosomes were G-banded (GTG) by Wright's or Giemsa staining (WANG, FEDOROFF 1972). Karyotypes were analysed and classified according to the ISCN (1995).

Eight selected tumors were examined in detail for the presence of abnormalities involving the short arm of chromosome 12, i.e. i(12p) and other markers carrying chromosome 12 material. G-banded metaphases with undefined markers were analysed subsequently by fluorescence in situ hybridization (FISH). For this purpose FISH with painting the whole chromosome 12 or its short arm was used.

Fluorescence in situ hybridization

The procedure outlined is based on that described originally by PINKEL et al. (1986). The following DNA probes were used: digoxigenin-labeled library probe for chromosome 12 (Coatasome 12, Oncor) and biotin-labeled library probe for the short arm of chromosome 12 (M28). The painting probes were denatured at 75°C for 10 or 5 minutes.

Slides for cytogenetic analysis were treated with RNase (RNase A, 100 mg/ml in 2 × SSC), washed in 2 × SSC, digested in pepsin solution (5 mg/100 ml 0,01M HCl), washed in PBS and then placed in 1% paraformaldehyde/1% $MgCl_2$ PBS. After this fixation step, slides were washed in PBS, dehydrated in alcohol series and left to dry. The dehydration preceded denaturation in 50% formamide/2 × SSC for 4 minutes at 75°C, after which the slides were placed in ice-cold 70% ethanol before being dehydrated again. Hybridization was carried out in a humidified chamber at 37°C overnight. Non-specifically bound probes were removed with three washes in 50% formamide/2 × SSC at 42°C (5 min.) and subsequently with three washes in 2 × SSC also at 42°C. Chromosomes and interphases hybridizing to biotin- or digoxigenin-labelled probes were visualised using alternating layers of fluorescein isothiocyanate (FITC)-conjugated avidin and biotinylated goat anti -avidin antibodies (both from Vector Laboratories, Burlingame, CA), or FITC-conjugated mouse anti-digoxigenin antibodies (Sigma) and FITC-conjugated goat anti-mouse antibodies (Jackson ImmunoResearch, West Grove, PA), respectively. Counterstaining of the chromosomes and interphases was performed with the blue DNA-specific dye DAPI (Sigma).

Chromosomes were studied under a Zeiss Axioskop epifluorescence microscope, equipped with appropriate filters for visualisation of FITC, Texas Red and DAPI fluorescence, as well as for simultaneous visualisation of propidium iodide and FITC fluorescence. Photographs were taken on Kodak Gold 400 colorslide film.

Results

Among the observed fifteen TGCTs, twelve tumors were kariotypically abnormal (Table 1). These cases showed a neartriploid or neartetraploid number of chromosomes per cell.

Table 2 presents the karyotypes of nine conventionally (G-banding) analysed cases, selected for FISH examination. Only three tumors in this group displayed the common i(12p) marker. The remaining five tumors lacked i(12p), but carried other numerous rearrangements. Cells analysed by banding showed structural

Table 1. Summary of clinical and cytogenetical G-banding data on TGCTs

Patient number	Patient age	Histology	Modal number	i(12p)	Unidentified markers
197	20	NS	54-56	+	+
199	36	NS	69-77	+	+
202	40	NS	52-60	-	+
203	45	NS	52-59	-	+
204	41	NS	60-64	-	+
208	27	SE	54-60	n.r.	n.r.
210	31	NS	61-66	+	+
214	31	SE	50-54	-	+
215	45	NS	~100	-	+
216	45	SE	46	-	-
217	43	SE	46	-	-
218	39	SE	90-100	-	+
219	35	SE	46	-	-
220	51	NS	56-69	n.r	n.r.
221	43	NS	47-50, 80-90	-	+

NS = nonseminoma
SE = seminoma
n.r. = no results

Table 2. Karyotypic description of eight cases selected for FISH analysis

Patient number	Composite karyotype
197	54-56, XY, -1, -2, -4, -5, der(5), -6, -7, -8, -10, -11, der(11), -13, -16, -17, -18, -19, -20, -21, -22, -22, **+i(12p)** × 1~2, +2mar
199	69-77, XXXYY, -4, +6, -19, -20, +21, **+i(12p)**, +5mar
203	59, XXY, -3, -4, -5, -6, -7q+, -9, -10, -11, **dup(12p)**, -13, -14, -16, -17, -18, -19, -20, -21, -22, +mar1, +mar2, +mar3, +2mar
204	63, XY, -2, -4, -5, -6, -8, -9, -11, -13, -14, -15, -16, der(16)t(1;16), -18, -22, +mar1, +4mar
208	hypotriploid
210	65, XXYY, +7, -10, +12q+, **+del(12q)**, -13, -14, -17, -20, -21, +22, + **i(12p)** × 1~2
215	hypertetraploid
220	hypotriploid
221	56-69, XY, -3, -4, -5, +7, -13, -14, -15, -15, -18, -21, -22, +mar1, +mar2, +mar3

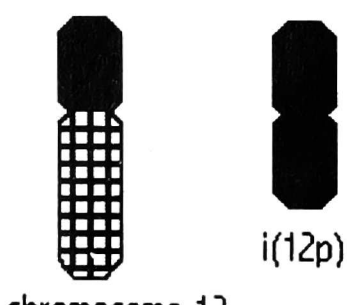


Figure 1. Ideogram of FISH using the painting probe of whole short arm of chromosome 12 (M28) on normal chromosome 12 and i(12p) marker. Black area shows 12p material, chequered area

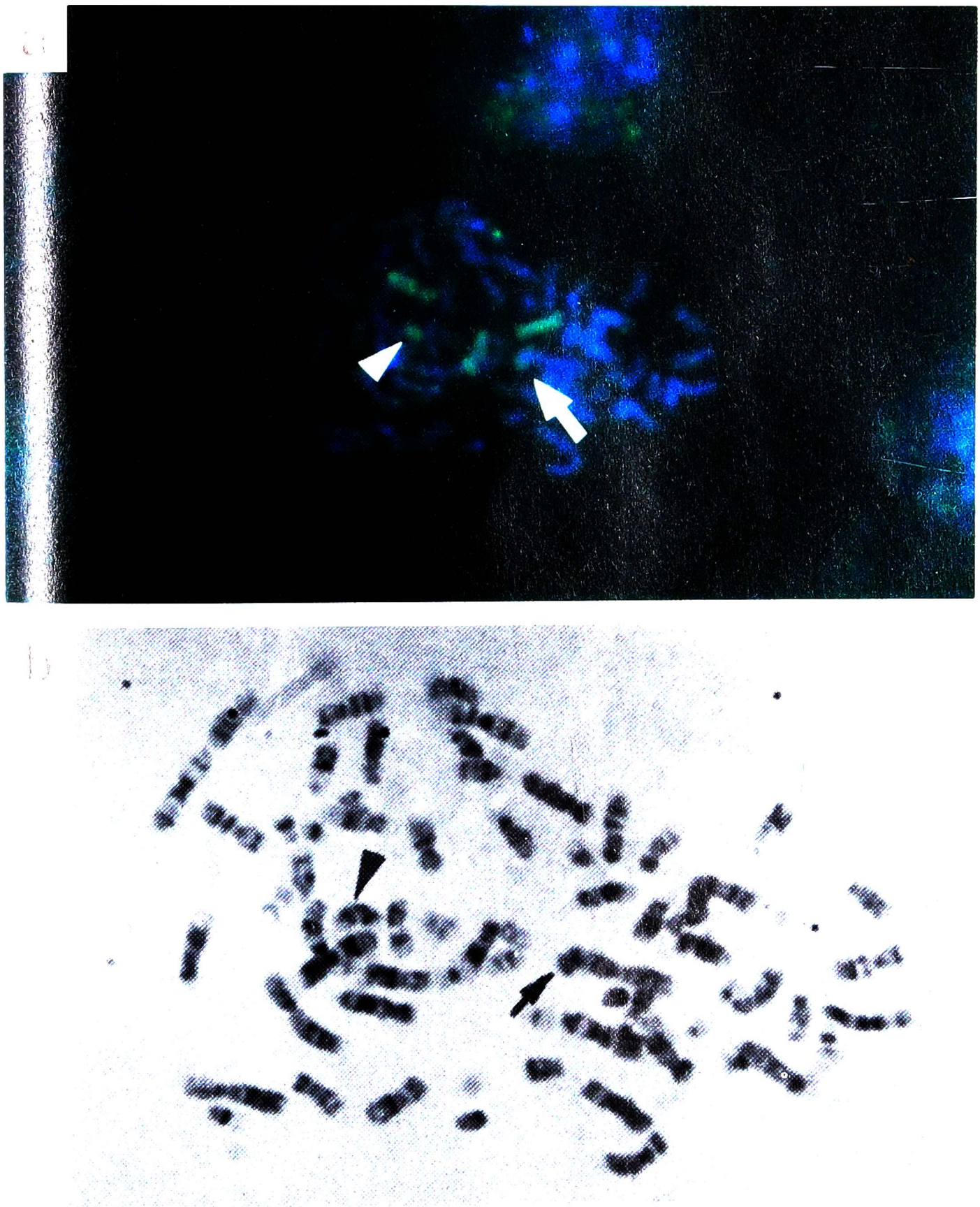


Figure 2. Results of FISH (a) after G-banding analysis (b) with a whole chromosome 12 (p-12) painting probe indicates three copies of a normal chromosome 12, i(12p) and marker 2 contains chromosome 12 material from patient 210. The arrowhead indicate i(12p), while the arrow shows marker 2.

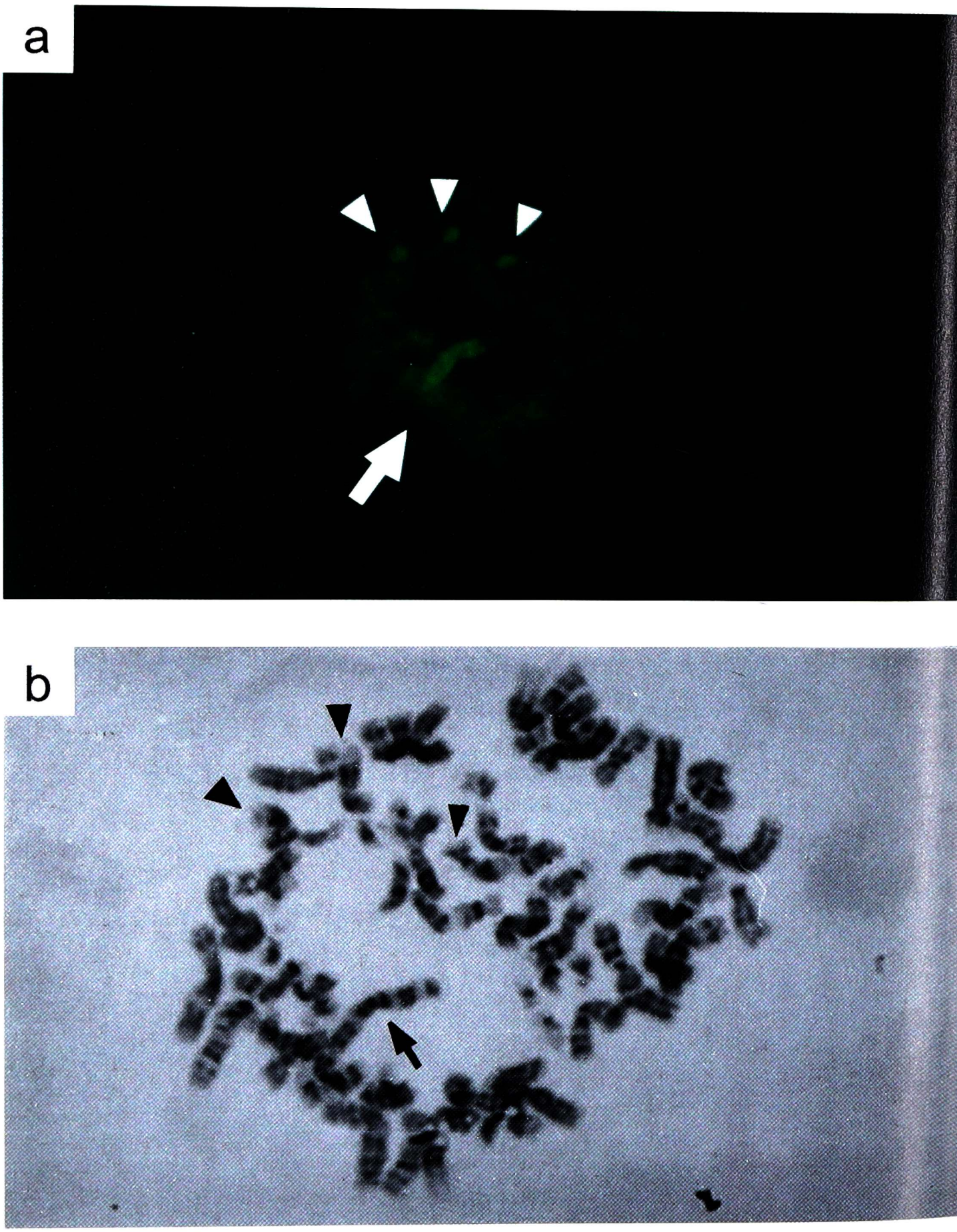
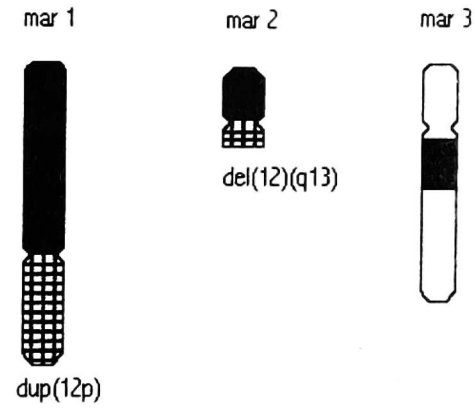
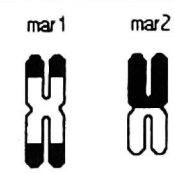
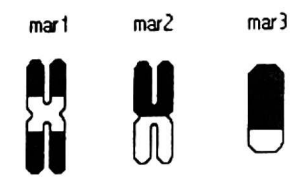


Figure 3. Results of FISH (a) after G-banding analysis (b) with a chromosome 12 short arm painting probe (M28) indicate three copies of normal chromosome 12 and long marker with amplification of 12p material from patient 204. Arrowheads indicate normal chromosomes 12, while the arrow shows a long marker with chromosome 12p amplification.

Table 3. Overrepresentation of 12p material detected by G-banding and FISH method. Ideograms present markers other than i(12p) detected by FISH. Black area shows 12p material, chequered area shows 12q material

Patient number	GTG i(12p)	GTG all unidentified markers	FISH the highest number of i(12p)	FISH markers carrying 12p material
197	1	2	2 × i(12p)	none
203	–	5	none	
204	–	5	none	
208	no results	no results	3 × i(12p)	none
210	1	1	2 × i(12p)	
215	no results	no results	2 × i(12p)	none
220	no results	no results	3 × i(12p)	
221	1	3	none	

anomalies involving chromosomes 1, 5, 11, 12, 16 and several unidentified markers. Two of these anomalies seemed to carry a cryptic rearrangement of chromosome 12: dup12p marker in case 203 and del(12q) in tumor 210 (Table 2). Generally, chromosome 12 rearrangements were found in only four of nine conventionally analysed cases.

Table 3 summarises chromosome-12-derived markers identified using FISH techniques and after G-band examination in a group of eight successfully hybridized tumors. The FISH analysis revealed that all eight tumors exhibited abnormalities, involving the chromosome-12-derived material. Therefore, the common i(12p) marker was found in five cases. Isochromosome i(12p) was detected as a sole rearrangement of chromosome 12 in three (197, 208, 215) of these tumors (see also Figure 1). In two further i(12p)-positive tumors (210, 220) cryptic rearrangements of 12p were detected additionally to i(12p) anomalies of chromosome 12 (see also Figure 2). In three remaining, i(12p)-negative tumors (203, 204, 221), cryptic rearrangements were recognised as structural anomalies involving chromosome 12-derived material (see also Figure 3).

As a result of subsequent FISH and GTG analysis, i(12p) markers and/or 10 other markers carrying 12p-derived material were demonstrated in all analysed cases (Figure 1 and Table 3). All these abnormalities included 12p-derived material (black areas) and (in some cases) 12q-material (chequered areas).

Discussion

Chromosome arm 12p rearrangements, including i(12p) and other markers, have been observed in virtually all primary testicular germ cell tumors (TGCT) examined by FISH (SUIJKERBUIJK et al. 1991, SMOLAREK et al. 1995, HOULDSWORTH et al. 1997).

We compared the results of conventional karyotyping and FISH in search of rearrangements of chromosome 12. A common i(12p) marker and a variety of other markers carrying chromosome 12p material was found in a selected group of eight TGCTs. The i(12p) marker (amplification of the whole short arm of chromosome 12) was visualised both by conventional analysis (GTG) and by FISH methods. However, most of the other 12p markers remained undefined following conventional cytogenetic banding.

Application of both methods of analysis demonstrated an overrepresentation of 12p derived material in all examined TGCT cases. At least one copy of i(12p) was found in five of the eight cases studied. These results are consistent with the previous data demonstrating the presence of i(12p) in over 80 per cent of all TGCTs (ATKIN, BAKER 1982, PIEŃKOWSKA et al. 1997).

Approximately 20% of testicular tumors do not have i(12p) but do contain other rearrangements of 12p (GIBAS et al. 1986). In our material in all three i(12p)-negative tumors, a variety of 12p abnormalities were revealed. These ab-

normalities could arise as a result of duplication of a part of 12p arm on chromosome 12; insertion of 12p material into other chromosomes; transfer of 12p-material to distal parts of markers; or whole 12p arm translocation. Thus, even in i(12p)-negative tumors overrepresentation of 12p-material was observed. The overrepresentation of 12p-derived sequences in i(12p)-negative TGCTs is probably analogous to one or more copies of i(12p) in i(12p)-positive TGCTs and may be essential for TGCT development (GIBAS et al. 1986).

Moreover, additional anomalies of 12p were detected in two i(12p)-positive tumours. Appearance of 12p-markers was similar in i(12p)-negative and i(12)-positive tumors. Our observation of the coexistence of i(12p) with other markers carrying chromosome 12p material is consistent with the results of SUIJKERBUIJK (1993), who described extra aberrations of chromosome 12, not only in i(12p)-negative, but also in i(12p)-positive TGCT (SUIJKERBUIJK et al. 1993).

As a result of combining data from FISH experiments with those from conventional chromosome banding analyses, other aberrations involving the short arm of chromosome 12 were identified in this study. The application of FISH technique with both types of probes (for the whole chromosome 12 or its short arm) considerably increased the detectability of chromosome 12 rearrangements in TGCT.

The short arm of chromosome 12 demonstrated a relative overrepresentation as compared with their modal chromosome constitution (neartriploid/neartetraplid) in the analysed group of tumors. It is well known that the amplification of whole chromosomal arms remains rather infrequent in tumorigenesis. The ubiquitous amplification of the whole short arm and/or part of 12p in germ cell tumors suggested that 12p arm carries gene(s) important for oncogenesis of male germ cell tumors. Recent studies by FISH using microdissection probes for specific bands of 12p have led to the hypothesis that 12p harbours more than one gene important for TGCT oncogenesis, which may be located in different areas of 12p (HENEGARIU et al. 1998). These sequences, disseminated along the short arm of chromosome 12, may play a role in tumor development, tumor growth advantage, and/or therapy resistance (MOSTOFI et al. 1987). The consistent overrepresentation of 12p sequences, by i(12p)-formation or other aberrations of chromosome 12, indicate that the gene(s) on the short arm of chromosome 12 play an essential role in TGCT oncogenesis. However, the relevant genes need to be identified.

REFERENCES

ATKIN N.B., BAKER M.C. (1982). Specific chromosome change, i(12p) in testicular tumors? Lancet 336: 183.

CASTEDO S.M., OOSTERHUIS J.W., de JONG B. (1991). Cytogenetic studies of testicular germ cell tumors: pathogenetic relevance. In: Recent Results in Cancer Research (Oosterhuis J.W., Walt H., Damjanow I., eds.). Springer-Verlag, Berlin 123: 101-106.

De GRAAFF W.E., OOSTERHUIS J.W., de JONG B., DAM A., VAN-PUTTEN W.L.J., CASTEDO S.N.M.J., SLEIJFER D.T.H., KOOPS H.S. (1992). Ploidy of testicular carcinoma in situ. Lab. Invest. 66: 166-168.

De JONG B., OOSTERHUIS J.W., CASTEDO S.M., VOS A., MEERMAN G.J. (1990). Pathogenesis of adult testicular germ cell tumors: A cytogenetic model. Cancer Genet. Cytogenet. 48: 143-167.

EL-NAGGAR A.K., JAE Y.R.O., MCLEMORE D., AYALA A.G., BATSAKIS J.G. (1992). DNA ploidy in testicular germ cell noeplasms: Histogenetic and clinical implications. Am. J. Surg. Pathol. 16: 611-618.

GEURTS-VAN-KESSEL A., SUIJKERBUIJK R.F., SINKE R.J., LOOIJENGA L., OOSTERHUIS J.W. (1993). Molecular cytogenetic of human germ cell tumors: i(12p) and related chromosomal anomalies. Eur. Urol. 23: 23-28.

GIBAS Z., PROUT G.R., PONTES J.E., SANDBERG A.A. (1986). Chromosome changes in germ cell tumors of the testis. Cancer. Genet. Cytogenet. 19: 245-252.

HENEGARIU O., VANCE G.H., HEIBER D., PERA M., HEEREMA N.A. (1998). Triple-color FISH analysis of 12p amplification in testicular germ-cell tumors using 12p band-specific painting probes. J. Mol. Med. 76: 648-55.

HOULDSWORTH J., REUTER V., BOSL G.J., CHAGANTI R.S. (1997). Aberrant expresion of cyclin D2 in early event in human male germ cell tumorogenesis. Cell Growth Diff. 8: 293-299.

ISCN (1995). An International System for Human Cytogenetic Nomenclature. (Mitelman F. ed). Basel: Karger (in collaboration with Cytogenet. Cell Genet.).

MOSTOFI F.K., SESTERHENN I.A., DAVIS C.J. Jr. (1987). Immunopathology of germ cell tumors of the testis. Sem. Diagn. Pathol. 4: 320-341

MURTY V.V.V.S., CHAGANTI R.S.K. (1998). A genetic prospective of male germ cell tumors. Sem. Oncol. 25: 133-144.

OOSTERHUIS J.W., CASTEDO S.M., de JON G.B., CORNELISSE C.J., DAM A., SLEIJFER D.T., SCHRAFFORDT KOOPS H. (1989). Ploidy of primary germ cell tumors of the testis. Pathogenetic and clinical relevance. Lab. Ivest. 60: 14-20.

PIEŃKOWSKA B., WITKOWSKA A., RYGIER J., GRYGALEWICZ B., BREGUŁA U. (1997). Analiza kariotypowa nowotworów jadra u człowieka. (Karyotypic analysis of human testicular tumors). Nowotwory 47: 108-119.

PINKEL D., STRAUME T., GRAY J.W. (1986). Cytogenetic analysis using quantiative high sensitivity fluorescence hybridization. Proc. Natl. Acad. Sci. USA 83: 2934-2938.

SMOLAREK T.A., BLOUGH R.I., FOSTER R.S., ULBRIGHT M., PALMER C.G., HEEREMA N.A. (1995). Identification of multiple chromosome 12 abnormalities in human testicular germ cell tumors by two-colour fluorescence in situ hybridization (FISH). Genes Chromosome Cancer 14: 252-258.

SKAKKEBAEK N.E., BERTHELSEN J.G., GIWERCMAN A., MULLER J. (1987). Carcinoma-in-situ of the testis: Possible origin from gonocytes and precursor of all types of germ cell tumours except spermatocytoma. Int. J. Androl. 10: 19-28.

STAMP I.M., JACOBSEN G.K. (1995). Infant intratubular germ cell neoplasia. Am. J. Surg. Pathol. 19: 489.

RODRIGUEZ E., MATHEW S., REUTER V., ILSON D.H., BOSL G.J., CHAGANTI R.S. (1992). Cytogenetic analysis of 124 prospectively ascertained male germ cell tumors. Cancer Res. 52: 2285-2291.

SUIJKERBUIJK R.F., SINKE R.J., MELONI A.M., PARRINGTON J.M., van ECHTEN J., de JONG B., OOSTERHUIS J.W., SANDBERG A.A., GEURTS van KESSEL A. (1993). Overrepresentstion of chromosome 12p sequences and karyotypic evolution in i(12p)-negative testicular germ-cell tumors revealed by fluorescence in situ hybridization. Cancer Genet. Cytogenet. 70: 85-93.

SUIJKERBUIJK R.F., LOOIJENGA L., de JONG B., OOSTERHUIS J.W., CASSIMAN J.J., GEURTS van KESSEL A. (1992). Verification of isochromosome 12p and identification of other chromosome 12 aberrations in gonadal and extragonadal human germ cell tumors by bicolor double fluorescence in situ hybridization. Cancer Genet Cytogenet. 63: 8-16.

SUIJKERBUIJK R.F., van de VEEN A.Y., van ECHTEN J., BUYS C.H., de JONG B. (1991). Demonstration of the genuine iso-12p character of the standard marker chromosome of testicular germ cell tumors and identification of further chromosome 12 aberrations by competitive in situ hybridization. Am. J. Hum. Genet. 48: 269-273.

WANG H.C., FEDOROFF S. (1972). Banding in human chromosomes treated with tripsin. Nat. New. Biol. 235: 52-53.