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Triple-color FISH analysis of 12p amplification in testicular germ-cell tumors using 12p band-specific painting probes

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Abstract Forty-nine surgical specimens and nine germ cell tumor lines were analyzed by triple-color FISH using microdissected probes for the cytogenetic bands of chromosome arm 12p (12p11.2, p12, and p13). FISH analysis demonstrated amplification of material from all three bands in all tumors. This amplification was in the form of increased copy number of 12p or i(12p) and/or 12p amplified regions (AMP12p). The number of copies of 12p was variable (4–11 copies) from case to case but tended to remain relatively constant in all clones of the same tumor, even when the amplification took the form of an amplified region composed of 12p material. In tumors with multiple clones, i(12p) and AMP12p were never found in the same cell. No correlation was found between 12p copy number and tumor type. We describe, for the first time, a relative overrepresentation of 12p13 or 12p12–p13 regions in six tumors (two surgical samples and four cell lines), either as "partial 12p" (five cases) or within a 12p amplified region (one case). The ubiquitous amplification of all three 12p bands in germ-cell tumors supports the hypothesis that 12p harbors more than one gene important for oncogenesis of adult male germ-cell tumors, and that these genes may be located in different areas of 12p.

Key words Germ-cell tumors · Testicular cancer · Multicolor FISH · Chromosome 12p · Gene amplification

Abbreviations *CGH* Comparative genomic hybridization · *FISH* Fluorescence in situ hybridization · *GBR* Green-blue-red color sequence · *GCT* Germ-cell tumors · *PCR* Polymerase chain reaction · *RBG* Red-blue-green color sequence

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Introduction

Amplification of chromosome arm 12p material in the form of one or more i(12p) can be detected in 70–80% of all human adult male germ-cell tumors (GCT), thus representing the most consistent chromosomal abnormality in these tumors [1–3]. The i(12p) characteristic of adult male GCT is not found in prepubertal GCT and is found only rarely in other types of tumors [4]. Patients with primary mediastinal GCT occasionally have an acute myeloid leukemia-like disease, and the presence of i(12p) in some of

these acute myelocytic leukemias led to their identification as germ cell in origin [5, 6]. In addition, adult GCTs are almost always hyperdiploid and frequently nearly triploid or tetraploid and may have other structural rearrangements (deletions, translocations) of chromosome 12 and of many other chromosomes [2, 7–12].

Approximately 20% of GCT are i(12p) negative. However, no significant difference has yet been found in clinical or biological parameters between patients with i(12p) negative or i(12p)-positive GCT [12–15]. Whereas classical cytogenetics (banded chromosomal analysis) readily detects the presence of an i(12p) and of many numerical and structural abnormalities, fluorescence in situ hybridization (FISH) technology has made it possible to identify 12p amplification in all i(12p)-negative adult GCT [11, 13, 15–18]. The ubiquitous presence of 12p amplification in GCT, even in carcinoma in situ, suggests that a gene(s) involved in the initiation and/or development of germ cell neoplasia is located on this chromosome arm.

Recently, using comparative genomic hybridization (CGH), a technique allowing detection of chromosomal gains and losses throughout the genome, an amplified subregion of chromosome arm 12p (12p11.2–12p12.1) was identified in five primary adult GCT and in the metastasis of one seminoma [19–21]. This finding may indicate that at least one gene involved in tumorigenesis or metastasis is located in this subregion.

In order to identify new tumors with limited amplified region(s) of 12p in GCT we performed triple-color FISH analysis of 49 metastatic and/or primary tumors and 9 cell lines [22, 23] using band-specific probes for 12p11.2–12.1, 12p12 and 12p13 obtained by microdissection. Our results indicate that all tumors have amplification of all three 12p bands in the form of i(12p), amplified regions comprised of multiple 12p copies (AMP12p), or as 12p material integrated into other chromosomes, with some tumors showing a relative overamplification of the distal part of 12p (12p12–p13).

Materials and methods

Specimens

Fresh tumor samples were collected over a period of approximately 3 years from patients treated at Indiana University. Also included in this FISH analysis were three cell pellets (samples 1–3) stored for 5–6 years (Table 1). The majority of the surgical samples analyzed were of metastatic origin, usually lymph nodes (Table 1). Specimens analyzed included: 36 teratomas (T), 7 mixed GCTs, 3 embryonal carcinomas, 1 yolk sac tumor, 1 tumor of uncertain origin (necrotic tumor), 1 acute myelocytic leukemia sample, and 9 GCT lines [22, 23]. Clinical data for 36 of the cases showed that all but four patients received chemotherapy prior to surgery. Three cases (6, 11, and 22) were diagnosed with relapsed GCTs.

Slide preparation, pretreatment, and denaturation

Short-term (1–14 days) cell culture was performed as previously described [13]. Slides were prepared as usual and were pretreated and hybridized the same day. To "age" the specimens, each slide was covered with ethanol and then placed on a heated metal block at 94°C for up to 2 min. This was followed by 1–2 min of 0.005% pepsin pretreatment and dehydration in an ethanol series. Slides were denatured in 70% formamide/2xSSC at 74°C, for 2 min, followed by ethanol dehydration.

Probe preparation and labeling

Probes corresponding roughly to the three 12p bands (12p11.2–12.1, 12p12, and 12p13) were obtained by microdissection (courtesy of Drs. Michael Bittner and Jeffrey Trent). The three probes were provided as mixtures of polymerase chain reaction (PCR) products obtained after amplification of the microdissected chromosome fragments using a degenerate universal primer previously described [24]. The three probes represent three roughly equal parts of 12p, with a degree of overlap at the borders (Fig. 1a–d).

These PCR products were reamplified and labeled by the same degenerative oligonucleotide priming PCR procedure, in which the 200 µM dTTP was replaced by a mixture of 133 µM dTTP plus 67 µM labeled dUTP. Three types of labeled nucleotides were used: digoxigenin 11-dUTP (Boehringer) for the 12p11.2–12.1 band probe, biotin 11-dUTP (Enzo) for the 12p12 band probe, and dinitrophenyl 11-dUTP for the 12p13 band probe.

Fluorescence in situ hybridization

For each hybridization 400–600 ng of each labeled probe DNA was mixed with 30–50 µg human Cot-1 DNA (Gibco), ethanol precipitated, resuspended in hybridization buffer, and denatured. The probe cocktail was placed on the previously denatured slides and hybridized overnight in a moist chamber at 37°C. After hybridization slides were washed in 0.1xSSC at 65–67°C and then subjected to antibody detection at the dilution recommended by the vendor (usually 100–200× in 2xSSC). All antibody incubations were performed at 37°C for 8–10 min and were followed by 3×5 min washes in 4xSSC/0.1% Tween 20 solution at 45°C.

The following antibodies were used in the detection protocol (Table 2): mouse anti-digoxigenin (Sigma); sheep anti-mouse Cy3 (Accurate); donkey anti-sheep Cy3 (Accurate); avidin D AMCA (Vector); mouse anti-avidin biotin (Sigma); rabbit anti-dinitrophenyl (Molecular Probes); goat anti-rabbit FITC; donkey anti-goat DTAF (Accurate). To avoid possible interactions the antibodies were combined in five detection steps/layers, as described in Table 2. Chromosomes were not counterstained as the fluorescence of both DAPI and propidium iodide would have interfered with the AMCA and Cy3 fluorescence.

FISH images were analyzed microscopically using an Aristoplan fluorescence microscope (Leitz) equipped with appropriate filters, and using a 100× oil-immersion objective. Images were captured with a cooled charged-coupled device camera (Photometrics) and a software package developed by Vysis. Separate gray images of the three probes (red, green, and blue) were taken using individual FITC, rhodamine and DAPI filters, transferred to a Macintosh computer, pseudocolored, and superimposed using a commercial software package (Vysis) to give the final image.

FISH data recording and analysis

One or two hybridizations were performed for each tumor analyzed. In each case the hybridization area was scanned in its entirety, using a 50× or 63× objective, and all abnormal metaphases were recorded (between 1–20). In each hybridization nuclei were analyzed as well, and some nuclear images were captured.

Table 1 Results of triple-color FISH on GCT (*AMP12p:* 12p amplicons, *Orig:* specimen origin, *Type:* tumor type, *AML:* acute myelocytic leukemia, *T:* teratoma, *M:* mixed GCT, *YST:* yolk sac tumor, *EC:* embrional carcinoma, *N:* necrosis, *LN:* lymph node, *Tst:* testis, *Lg:* lung, *K:* kidney, *G*, *B:* partial chromosome 12p fragments, hybridizing with only the green or the blue paint probe)

	i(12p)	12p-like	part 12p	AMP12p	Orig	Type/name
Surgical specimens						
1		4 Partial		1	Tst	T
2		4			LN	EC
3		4			Tst	M
4	2	3			LN	EC
5	2	7			LN	T
6		3		1	LN	T
7	1	4			LN	M
8		4–5			LN	M
9		4			LN	T
		5				
		4				
10		8		1	LN	EC
	5–6			1		
	1	4				
11	1	4			LN	T
12	1	4			LN	T
	1	2				
		2		1		
13	2	3			LN	T
14		4			LN	T
		3		1		
		2		2		
15	1	3			LN	T
	2	3				
16	1	3			LN	T
17	1	3			Tst	M
	2	3				
18	3	3			LN	T
	3	4				
19	1	3	1G		LN	T
	1	4				
20	1	4			LN	T
21	2	4			LN	T
		3		1		
22		6			LN	T
23	1	5			LN	T
	2	10				
24	1	4			LN	T
25	1	3			Lg	T
	2	3				
26	1	3			LN	T
27	2	2			LN	T
28	2	2			LN	T
29	1	4			LN	T
30	1–3	2–3			LN	T
31	1	4			LN	T
32	1	3			LN	T
33	1	2			LN	T
	1	3				
34	1	2			LN	AML
35	1	3			Tst	M
36	1	2			Lg	T
	3	4				
37	1–2	5			LN	T
38	3	2			Lg	T
39	2–3	5			LN	YST
40	3	3			K	T
41	3	3			Tst	M
42	1	3			LN	T
43		4			Md	T
44	2	5			LN	T
45	1	2			LN	N
46	2	3			Tst	M
47	2	3			LN	T
	3	5				
48	1	5			Lg	T
		5–6		1		
49	1	5			LN	T
GCT cell lines						
1	2	3	3 GB			833 K
	1	4–6				
2		4				1411H
3	1	4–5	2 GB			N2102
	2	3	1 GB			
4	1	3				NT2D1
5		6				44
6	1	2				72
7	2	3	1 GB			27X-1
8	3 (2–6)	3 (2–4)				27C4
9	3	3				63

Table 2 Antibody detection protocol

	Biotin	Digoxigenin	Dinitrophenyl
Step I	Avidin AMCA[a]	Mouse anti-digoxigenin	–
Step II	Goat anti-avidin biotin[a]	Sheep anti-mouse Cy3	–
Step III	Avidin AMCA	Donkey anti-sheep Cy3	Rabbit anti-dinitrophenyl
Step IV	Mouse anti-avidin biotin	–	Goat anti-rabbit FITC
Step V	Avidin AMCA	–	Donkey anti-goat DTAF

[a] Optional antibodies, when AMCA signals needed amplification

Results

The 12p11.2–12.1 probe was colored red-orange, the 12p12 probe was blue, and the 12p13 probe was green (Fig. 1a–d). Therefore normal 12p signals had the color sequence red-blue-green (RBG) or green-blue-red (GBR). The three microdissected probes slightly overlap at their borders. The 12p12 probe also hybridizes weakly to the X chromosome centromere and the Y chromosome heterochromatin (Fig. 1d). In very good preparations, the 12p13 and 12p12 probes hybridize weakly to the centromeres of two other C-group chromosome pairs (Fig. 1a–d). These unspecific signals were partly visible in a few hybridizations and were clearly weaker than the 12p specific signals and did not interfere with the interpretation of the results.

Triple-color FISH analysis on tumor specimens identified four types of hybridization patterns/signals:

– "12p", comprised of sequential signals for the three bandprobes used. FISH using painting probes has limitations in resolution. Therefore signals called 12p need to be interpreted with caution, as small deletions in the three bands may escape detection. As no counterstain was used, it was not always apparent whether 12p signals belonged to the p arm of a normal chromosome 12 or to a 12p translocated or inserted in a different chromosome.

– "i(12p)", with a color sequence GBR-RBG (Fig. 1i–l).

– "AMP12p", which were long amplified regions containing two or more copies of 12p material located on the same chromosome (Fig. 1m–p). Interestingly, with one exception (sample 48), all amplified regions were made of multiple 12p units (i.e., RBG sequence) arranged in all possible positions: head to tail, tail to head, tail to tail, or head to head. Two or three different orientations could be seen in the larger AMP12p but, with the one exception, the RBG sequence seemed to be preserved. The AMP12p of surgical sample 21 showed a relative overrepresentation of green and blue (12p13–12p12).

– "Partial 12p", containing signals representing the distal part of 12p, either 12p12 and 12p13 probes or only 12p13, but not as part of an AMP12p. Partial 12p was seen in five cases (Fig. 1q–t).

All cases showed overrepresentation of all three 12p bands. Based on the types of signals described above, the number of copies of 12p was counted in both the surgical specimens and the GCT lines. Each RBG color sequence was counted as one 12p copy and each i(12p) was counted as two 12p copies. The RBG color pattern was identified in each AMP12p (except sample 48) and the number of 12p units counted. Results are shown in Fig. 2. Occasionally, metaphases with three or four different hybridization patterns were found in a single tumor (multiple clones). Interestingly, although the number of 12p varied from case to case, in most cases all clones of the same tumor showed a similar number of 12p, even though the form in which 12p was amplified was quite different. Additionally, i(12p) and AMP12p were mutually exclusive in the same cell: although they were simultaneously present in some of the tumors, none of the clones contained both (Table 1).

Surgical samples

As depicted in Fig. 2a, the number of apparent 12p copies in the surgical samples varied from 4 to 11. No correlation between tumor type and 12p copy number was apparent. Eight surgical specimens (16%) were *i(12p) negative*, with 4–6 copies of 12p each. Three of the i(12p)-negative tumors showed two or three different clones; in each case one clone had one or two "AMP12p" (Fig. 1h–l). Of the surgical specimens 41 (84%) were *i(12p) positive*. Of these, 36 were "monoclonal" tumors, with one to three i(12p)s and many also had multiple copies of 12p (3–7 copies) elsewhere in the genome (Fig. 1m–o). Five of these specimens had both i(12p)-positive and i(12p)-negative clones (samples 6, 10, 12, 21, and 48). In each of these cases at least one of the i(12p)-negative clones had one or two AMP12p. Nine other i(12p)-positive tumors had two or three different clones, with various numbers of i(12p). Sample 19 had two clones, one with one i(12p) and four 12p and another with one i(12p), three 12p, and one limited 12p region (12p13) within another chromosome.

AMP12p structure

The pattern of the 12p bands on the "painted" AMP12p showed interesting variations. With the exception of case 48, all AMP12p showed two to five "12p units" in various orientations. These 12p units were either juxtaposed (Fig. 1m) or separated by non-12p material. For example, sample 14 had two clones with two AMP12p, of different sizes. The small amplified region appeared to be composed of two 12p units following the order RBG-GBR, whereas the large amplified region appeared to be a double of that pattern (a duplication of the smaller AMP12p). In sample 9 there was one amplified region containing two 12p units arranged in a head-to-tail fashion, separated by a small non-12p area, whereas another 12p unit was located within a larger marker chromosome.

An exception to the pattern of sequential 12p-band representation was sample 48, in which one of the clones had one i(12p) and five 12p, whereas a second clone had five 12p and one AMP12p in which the RBG pattern of amplification seemed disrupted (Fig. 1h). This amplified region consisted of two identical parts, joined palindromically. Size comparison of the signals indicated approximately equal representation of all three colors. Red signals were located near the ends of the amplified region, with blue and green signals between them. The red signals appeared to be somewhat larger than on a normal 12p. The blue and green signals were not distinct, as in other amplified regions but seemed to overlap. Moreover, in the middle of the blue-green block there was a thin band of red signal,

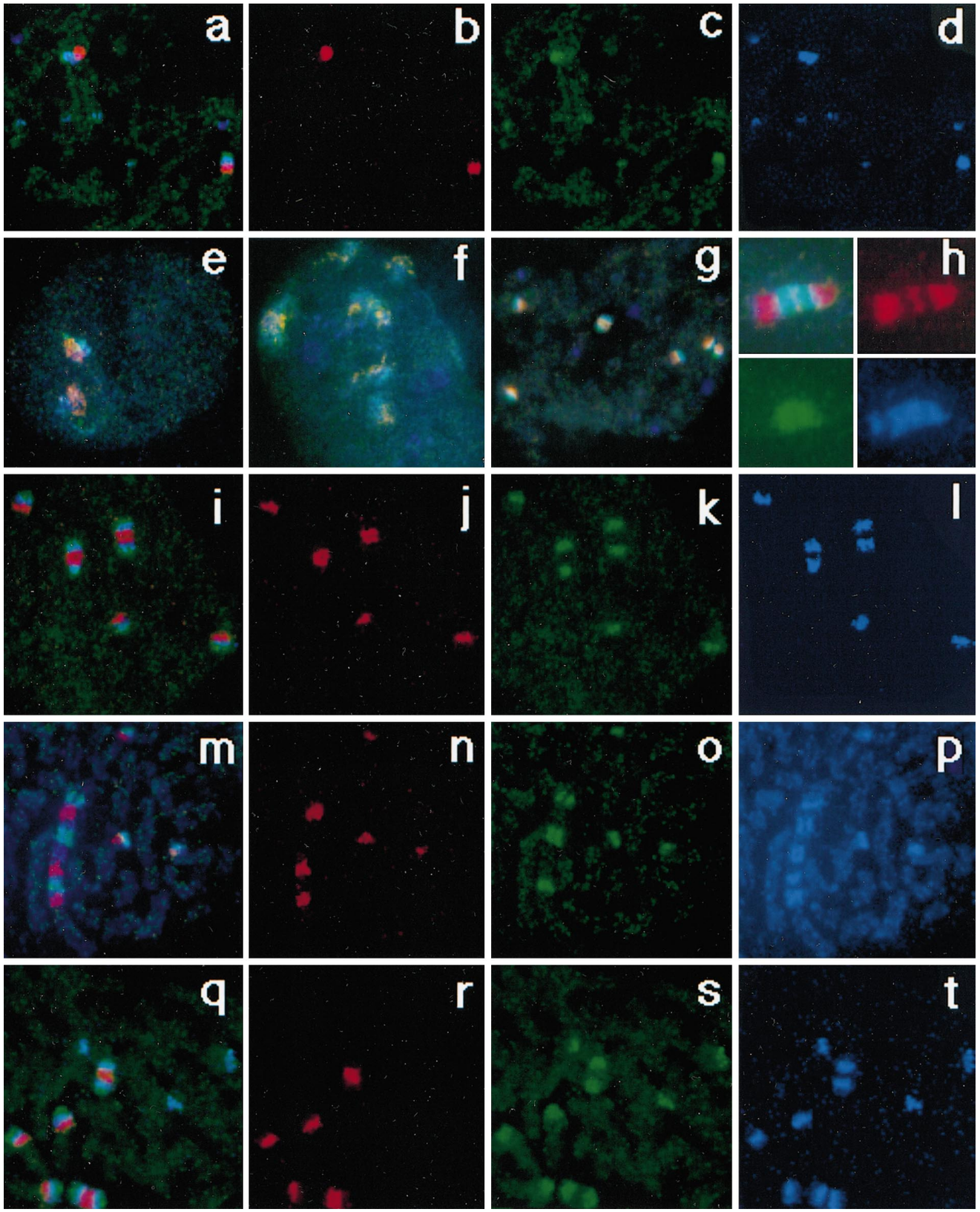

Fig. 1 For legend see page 653

◀ **Fig. 1a–t** Composite figure with triple-color FISH images of normal control and tumors with various numbers of 12p, i(12p), partial 12p, and AMP12p. **a–d** Hybridization of the three microdissected 12p probes to a normal metaphase, showing the 12p11.2–12.1 probe (*red*), 12p12 (*blue*), and 12p13 (*green*). An increased number of small blue and green signals is visible, due to the unspecific hybridization of the 12p12 probe to the chromosome X centromere and Y heterochromatin in some preparations and of the 12p12 and 12p13 probes to two group-C chromosomes. **b–d** The image was split into its three components to show hybridization of each probe. Chromosomes were not counterstained in these hybridizations. Metaphase shown was chosen for its shorter chromosomes, similar to the metaphases generally found in the tumors. **e** Hybridization to a normal nucleus. **f–g** Nucleus and metaphase of a clone from surgical sample 48. The nucleus showed six primary signals. One of the hybridization signals is roughly twice as large as the other ones, corresponding to the i(12p) in the metaphase depicted in **g**, which shows one i(12p) and five 12p (two of the 12p are very close to one another). The increased number of faint blue signals is due to the cross-hybridization of the 12p12 probe to the chromosome X centromere and Y heterochromatin in some preparations. **h** Structure of the amplified region of one of the clones of surgical sample 48. The composite image as well as the hybridization with each of the three different probes is shown. The amplified region is symmetric, with two red signals towards the ends and a thin red band in the middle. The blue color was located primarily between the red signals, with two thin stripes of blue visible outside the main block of material. The green signal was located primarily between the two red signals and was mixed with the blue color, with no apparent pattern. **i–l** Metaphase from sample 25 with two i(12p) and three 12p. **m–p** Amplified region in surgical specimen 21 showing five 12p copies, in the order (bottom to top): RBG-GBR-RBG-GBR-RBG. The distance between the top and the middle red signals is larger than the distance between the bottom and the middle red signals (*arrows*). It appears that there is an excess of green and blue between the top and the middle red signals, indicating a moderate overrepresentation of green and blue compared with the red. **q–t** Major clone of GCT line 833 K, with two i(12p), three 12p and three partial (green and blue only) 12p signals. The three GB signals are much stronger than the previously described possible cross-hybridization to the centromeres of some chromosomes (compare **a–d**), clearly indicating their 12p origin

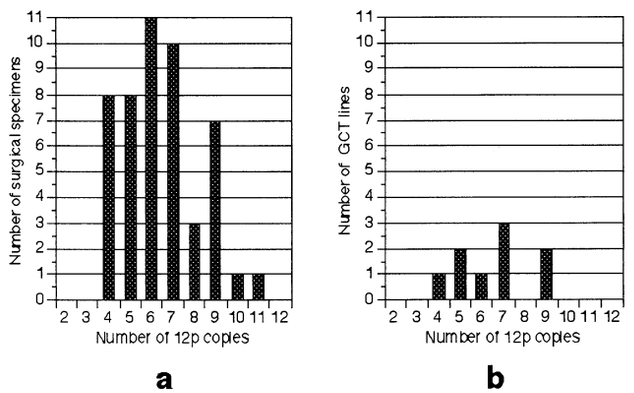

Fig. 2a,b Relative number of 12p copies. **a** Surgical samples. **b** GCT lines

dividing the amplified region into two symmetrical parts. When only the blue color was examined, there were two thin bands of blue fluorescence located outside the red signals, at the ends of the amplified region. The significance of these varying patterns is unknown but, whether in an orderly or disorderly fashion, material from all three 12p bands is represented in all AMP12p.

Distal 12p appeared overrepresented within the AMP12p of a surgical sample (no. 21). In this sample (Fig. 1m–p), one clone had three 12p and one amplified region with apparently five 12p-like units, in the bottom-to-top order RBG-GBR-RBG-GBR-RBG. However, in this amplified region the distance between the top and the middle red signals was almost twice as large as the distance between the middle and the bottom ones, indicating that the blue and green areas of 12p (12p12 and 12p13, respectively) between the middle and top red signals were somewhat overrepresented compared with the red band (12p11.2–12.1).

GCT cell lines

Nine GCT lines were analyzed by FISH, and, as in the surgical specimens, all showed amplification of all three 12p bands. The 12p copy number in each line varied from four to nine, with a distribution similar to the surgical specimens (Fig. 2b). Among these cell lines two were *i(12p) negative*, with four (1411H) or six (GCT 44) 12p copies in each cell. In line 1411H there was frequent polyploidy, with metaphases and nuclei with 8, 12, or 16 copies of 12p per cell. About 10% of all metaphases also showed two to four copies of partial 12p signals (blue and green signals). Seven cell lines were *i(12p) positive* (Fig. 1q–t); three had multiple clones (833K, N2102, and GCT 27C4).

Among the cell lines four (833K, N2102ep, 27X-1, and 1411H) showed a relative overamplification of the distal half of 12p (blue and green signals) compared with the proximal half. In 833K (Fig. 1q–t) the predominant clone had two i(12p), three 12p, and three partial 12p (blue-green only), and a subclone had one i(12p) and six 12p. Line N2102ep had one clone with one i(12p), five 12p, and two partial 12p (blue-green) and another clone with two i(12p), three 12p, and one partial 12p (blue-green only). Line 27X-1 had only one clone with two i(12p), three 12p, and one partial 12p (blue-green).

Discussion

The most common cytogenetic abnormality found in adult testicular GCTs is the overrepresentation of 12p sequences [1–3, 13, 15]. The 12p amplification may indicate that one or more (onco)genes located on this chromosome arm play an important role in the malignancy. To search for restricted regions of 12p amplification in adult GCT we performed triple-color FISH with painting probes for the three cytogenetic bands of 12p (Fig. 1) on 58 tumor samples (49 fresh specimens and 9 cell lines). For this study FISH was used rather than CGH as it provides more detailed information regarding the pattern of 12p amplification within each tumor genome and allows detection of different clones in each tumor. Although the majority of tumors studied were teratomas, previously published re-

ports did not detect any consistent cytogenetic differences between teratomas and other subtypes of GCT [10, 25]. Our results provide a number of novel observations regarding the pattern and the overall degree of 12p amplification in GCT.

Complete 12p amplification

All three 12p bands were amplified in all tumors analyzed; two surgical samples and four cell lines showed 12p12–13 overrepresentation (Fig. 1q–t). Although 12p amplification seems necessary for malignant transformation and/or progression, the 12p content of the malignant cells differs from one sample to another (Table 2). In this study 4–11 12p copies were found per tumor, with no apparent correlation between the tumor type and 12p copy number (Fig. 2). With the exception of sample 48 described above, even when AMP12p(s) were detected, the three painting probes appeared to be amplified as a unit, following a 12p-like color banding pattern (Fig. 1m–p).

All AMP12p analyzed showed two to five "12p units" in various orientations. The sequence and size of the three painting probes in each unit appeared unaltered (RBG), indicating that (almost) all of 12p was present in each individual unit, with no apparent over- or underrepresentation of any of the 12p bands (except sample 21).

Relative preservation of 12p copy number

Eighteen of the 49 surgical specimens and three of the nine GCT lines studied had various clones with variable 12p amplification patterns. In tumors with multiple clones i(12p) and AMP12p were never found in the same cell. Therefore it is possible that i(12p) and AMP12p are alternative forms in which the genes required for GCT pathogenesis are amplified, with i(12p) a much more frequent means of 12p amplification than AMP12p. As detailed in the results, the number of 12p copies tended to be maintained in all clones of a tumor but the reason for the apparent preservation of 12p copy number is not clear. Notable exceptions were samples 14, 25, 36, and 47 in which one clone had roughly twice the number of 12p copies of the other clone. It is possible that these clones represented polyploidization. No correlation between 12p copy number and tumor type was detected.

Distal 12p overrepresentation

As shown in Table 1, partial 12p sequences (12p12 and/or 12p13) were identified in one surgical specimen (no. 19) and four cell lines (833 K, N2102ep, 1411H, and 27X-1). Another surgical specimen (no. 21) showed distal 12p overamplification within its AMP12p.

It is not known whether the blue-green signals include the entire 12p12 and 12p13 bands or only some sequences from these two bands. As the two paint probes partially overlap, it is possible that the 12p region involved is located at the border of the two bands. These observations indicate that 12p12 and/or 12p13 (or their border) may harbor gene(s) important for the pathogenesis of GCTs. Previously published data showed proximal 12p (12p11.2–p12.1) to be overamplified in a few tumors [19, 21, 26], whereas our results indicate that distal 12p is overamplified in some GCT. These data together provide some evidence that, although the entire 12p is overrepresented in most GCT, further amplification of various sequences from 12p may provide some tumors with a proliferative advantage. These data also suggest that more than one 12p gene, when moderately overrepresented and presumably overexpressed, cooperate in tumorigenesis in adult male GCT. These genes may be located in two or more regions of chromosome arm 12p, possibly representing a novel mechanism of tumorigenesis.

Alternatively, as 4 of 9 cell lines but only 2 of 49 surgical samples showed distal 12p overrepresentation, it is also possible that there is either some bias in tumors capable of forming cell lines, or that this genetic change occurs more frequently in vitro.

Conclusion

In conclusion, triple-color FISH on 58 GCT samples showed amplification of the entire 12p or, at least, DNA from all three bands of 12p in all tumors. Our results showed for the first time that a distal 12p region is overrepresented in a limited number of tumors (6/58), that i(12p) and 12p amplified regions (AMP12p) do not coexist in the same cell and provided an insight in the architecture of the AMP12p. The number of copies of 12p was variable (4–11 copies) from case to case but tended to remain relatively constant in all clones of a tumor. These results support the hypothesis that all three bands of 12p are ubiquitously amplified in these tumors because more than one 12p gene is important for the pathogenesis of GCT, and that these gene(s) are located in different areas of 12p.

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