Virchows Arch (1996) 428:53–57

ORIGINAL ARTICLE

Jutta Jenderny · Eckard Köster · Olaf Borchers
Annette Meyer · Werner Grote · Dieter Harms
Ute Jänig

Interphase cytogenetics on paraffin sections of paediatric extragonadal yolk sac tumours

Received: 11 September 1995 / Accepted: 4 January 1996

Abstract Germ cell tumours in children are more often extragonadal than in adults and the most frequent type is the yolk sac tumour. Limited cytogenetic data exist on extragonadal yolk sac tumours in children. We applied in situ hybridization (ISH) to interphase cell nuclei of four paediatric extragonadal pure yolk sac tumours and one yolk sac tumour component of a mixed germ cell tumour using paraffin-embedded tissue sections. The panel of chromosome-specific DNA probes was selected on the basis of their relevance in adult germ cell tumours and consisted of five DNA probes specific for the (peri)centromeric regions of chromosomes 1, 8, 12, and/or 17, X and/or one DNA probe specific for the subtelomeric region of chromosome 1 (p36.3). Only one tumour failed to show numerical and structural chromosome aberrations with the DNA probes used. The other four had an increased incidence of numerical chromosome aberrations with an over-representation of at least one chromosome. The DNA indices determined in the paraffin-embedded tumour material correlated well with the in situ hybridization findings. In only a few cases were chromosomes over-represented, when compared with the corresponding DNA indices. Recently, we have shown that the short arm of chromosome 1 is a non-random site of deletion in paediatric gonadal pure yolk sac tumours. The occurrence of similar deletions in one extragonadal pure yolk sac tumour and in one yolk sac tumour component, in conjunction with two further ISH reports, suggests that the loss of gene(s) in this region is an important event in the pathogenesis of paediatric malignant germ cell tumours of nearly all sites.

J. Jenderny (✉) · O. Borchers · A. Meyer · W. Grote
Department of Human Genetics, University of Kiel,
Schwanenweg 24, D-24105 Kiel, Germany
Tel.: (49) 431-59 71 813; Fax: (49) 431-59 71 880

E. Köster
Department of Cytopathology, University of Kiel,
Michaelisstrasse 11, D-24105 Kiel, Germany

D. Harms · U. Jänig
Department of Paediatric Pathology, University of Kiel,
Michaelisstrasse 11, D-24105 Kiel, Germany

Key words Germ cell tumours · In situ hybridization · Paraffin-embedded tissue

Introduction

Germ cell tumours (GCTs) in children differ in many respects (epidemiology, clinical behaviour, distribution of sites and morphological subtypes) from GCTs in adults. In this age group the percentage of extragonadal tumours is higher than in adults. The majority of malignant childhood GCTs are either pure yolk sac tumours (YSTs) or mixed GCTs with a YST component [2, 3]. To our knowledge, only one cell line of a paediatric extragonadal pure YST [1] and four paediatric extragonadal pure YSTs or mixed GCTs with a YST component have been studied cytogenetically [9].

The aim of the study was to investigate whether paediatric extragonadal YSTs have similar chromosome aberrations to GCTs in adults. Paraffin sections of four paediatric extragonadal pure YSTs and the YST component of a mixed GCT from the Kiel Paediatric Tumour Registry were investigated for chromosome aberrations by in situ hybridization (ISH) with four DNA probes specific for the (peri)centromeric regions of chromosome 1, 12, and/or 17, X, as these chromosomes are non-randomly gained in adult male extragonadal GCTs. To relate the degree of aneuploidy to the GCT-associated chromosomes examined, we utilized the DNA probe for the centromeric region of chromosome 8, because gain of this chromosome has not been documented in adult patients [11]. A subtelomer-specific DNA probe was used to study deletions in the short arm of chromosome 1 at p36.3, since breaks in the short arm of chromosome 1 were common in adult male extragonadal GCTs [10, 11]. The DNA content was measured in isolated (intact) nuclei from the paraffin-embedded tumour material after Feulgen staining using an image analysis technique, in an attempt to validate the ISH findings by an independent method.

Materials and methods

We evaluated four extragonadal pure YSTs (KT 15/86, KT 442/89, KT 379/90, KT 302/77) and the YST component of an extragonadal mixed GCT (KT 144/91). Three GCTs were primary tumours and two were metastatic lesions. KT 442/89 was located in the pelvis, KT 379/90 and KT 144/91 in the coccyx. KT 302/77 was an extragonadal tumour of unknown origin and the metastasis was from the iliac crest. KT 15/86 was located in the retroperitoneum and the metastasis was from the liver. The mixed GCT KT 144/91 was composed of a dominant YST component and a smaller teratoma component. Sections (5 µm and 8 µm) were cut from archived formalin-fixed, paraffin-embedded tissue blocks and mounted on 3-aminopropyl-triethoxysilane (APES)-coated slides [7]. Five (peri)centromere-specific DNA probes for chromosomes 1 (D1Z1), 8 (D8Z2), 12 (D12Z3), 17 (D17Z1), X (DXZ1), and the subtelomer-specific DNA probe for chromosome 1 (D1S32, p36.3) were used. Pretreatment with 1 M sodium thiocyanate (NaSCN) and pepsin digestion of paraffin sections, description of DNA probes and labelling procedure were as described previously [6]. The evaluation and interpretation of ISH signals in paraffin sections were as follows. One paraffin section from each GCT was stained with haematoxylin and eosin counterstain to identify the tumour areas. For all DNA probes, the number of signals per nucleus was evaluated in at least 200 nuclei (maximum: 600). The ISH results were usually verified by a second observer (data not shown). The criteria for evaluating ISH signals established by Hopmann et al. [5] were followed. For DNA cytometry, single cell suspensions were prepared from 50-µm paraffin sections of each tumour [4] on silanized slides after using a bucket centrifuge (Shandon, Germany). Cell cycle and DNA ploidy analysis was performed on an IBM 486, AT computer with the cytometry analysis system AHRENS-ACAS (Bargteheide, Germany). Calculation of DNA indices (DIs) and definition of ploidy status were as described previously [6].

Results

In the present study, the incidence of hybridization signals per nucleus after ISH with the six DNA probes was evaluated in 5-µm paraffin sections of four extragonadal pure YSTs and one YST component of an extragonadal mixed GCT. Table 1 summarizes the number of hybridization signals per nucleus after ISH with the DNA probes used. Normal and stromal regions adjacent or in gonadal pure YSTs served as internal (diploid) controls. The corresponding incidence and distribution of control hybridization signals have been reported previously [6]. In summary, with all DNA probes examined, 35–43% of nuclei showed one hybridization signal, 49–58% two hybridization signals, and 0–1% three signals. At least 200 nuclei per DNA probe were counted in normal diploid tissues. Based on these data, an over-representation of a chromosome was assumed in tumour cells if the incidence of nuclei with three or more hybridization signals was above the standard value of 3.6% (the confidence interval for 1% of 200 analyzed nuclei is between 0.1% and 3.6%). With the exception of KT 15/86 all tumours showed an over-representation of at least one chromosome. A gain of chromosome 12 was observed in four tumours. For example, a trisomy and tetrasomy of chromosome 12 was assumed in tumour cells of KT 302/77 because more than 3.6% of nuclei showed up to four hybridization signals after ISH with the DNA probe D12Z3 (Tables 1, 2). An under-representation of a chromosome was assumed in tumour cells if less hybridization signals were observed compared with the copy number of chromosome 8 and to the corresponding DI. Such imbalances were only detected in gonadal YSTs. For example, a disomy of chromosome 1 was present in KT 429/90 with up to four hybridization signals with the DNA probe D8Z2 and with a near tetraploid DI of 1.96 (Table 2) [6]. Deletions in the short arm of chromosome 1 at p36.3 were detected in two of four extragonadal GCTs. For example, in KT 379/90 four hybridization signals with the DNA probe D1Z1 could be observed (Fig. 1). On parallel 5-µm paraffin sections only nuclei with three hybridization signals could be detected with the DNA probe D1S32, so that the total loss of one hybridization signal indicated a deletion at 1p36.3 in this tumour. All ISH results were verified by a second observer (data not shown) and, in KT 379/90, on thicker paraffin sections. Except for two extragonadal tumours (KT 144/91 and KT 302/77) all other tumours had abnormal DIs.

Table 1 Percentages of signals per nucleus in 5-µm and/or 8-µm paraffin sections of four extragonadal yolk sac tumour (KT 15/86, KT 442/89, 379/90, KT 302/77 and one extragonadal yolk sac tumour component (KT 144/91)

DNA probe	Section thickness	Number of signals per nucleus (%)				
		0	1	2	3	4
KT 144/911						
D1Z1	5 µm	3	24	63	10	0
D1S32	5 µm	3	56	41	0	0
D12Z3	5 µm	5	25	66	4	0
D17Z1	5 µm	6	29	60	5	0
D8Z2	5 µm	3	33	61	3	0
KT 15/86						
D1Z1	5 µm	6	43	50	1	0
D1S32	5 µm	7	50	43	0	0
D12Z3	5 µm	9	35	55	1	0
DXZ1	5 µm	8	39	53	0	0
D8Z2	5 µm	4	47	49	0	0
KT 442/89						
D1Z1	5 µm	9	31	46	13	1
D12Z3	5 µm	5	21	48	20	6
DXZ1	5 µm	3	27	59	10	1
D8Z2	5 µm	4	18	64	12	2
KT 379/90						
D1Z1	5 µm	2	10	35	37	16
	8 µm	1	8	31	41	19
D1S32	5 µm	3	31	54	12	0
	8 µm	1	26	67	6	0
D12Z3	5 µm	4	19	50	20	7
	8 µm	2	12	32	35	19
D17Z1	5 µm	0	9	37	38	16
DXZ1	5 µm	1	13	46	26	14
	8 µm	1	15	37	31	16
D8Z2	5 µm	3	17	45	26	9
	8 µm	1	8	33	31	27
KT 302/77						
D1Z1	5 µm	3	34	61	2	0
D1S32	5 µm	8	40	51	1	0
D12Z3	5 µm	5	32	59	3	1
D17Z1	5 µm	4	43	52	1	0
DXZ1	5 µm	5	25	68	2	0
D8Z2	5 µm	7	29	61	3	0

Table 2 Summary of in situ hybridization results obtained from 5-µm and/or 8-µm paraffin sections and DNA indices of 11 pure yolk sac tumours and one yolk sac tumour component (*YST* yolk sac tumour, [1] yolk sac tumour component, *P* primary tumour analysed, *Me* metastasis analysed, *F* female, *M* male, *NA* not analysed, *CR* complete remission, *LFU* lost of follow-up, *DOC* death of complication)

YSTs	Age	TNM-classification	Localization	Metastasis	Status	Maximum number of signals per nucleus after ISH with (peri)centromere-specific DNA probes of					DNA indices	Deletion at 1p36.3
						1	12	17	X	8		
KT 144/91P[1]/F	2 years	T2aNXMX	Coccyx	–	CR	3	3	3	NA	2	1.00	+
KT 15/86 Me/F	8 years	pTXNXM1	Retroperitoneum	Liver	?	2	2	NA	2	2	1.23	–
KT 442/89P/F	9 months	T2bNXM1	Pelvis	–	DOC	4	4	NA	4	4	1.45	NA
KT 379/90P/F	8 months	T2bNXM1	Coccyx	–	CR	4	4	4	4	4	1.92	+
KT 302/77 Me/F	17 years	pTXNXM1	?	Iliac crest	?	2	4	2	2	2	1.00	–
KT 381/87P[a]	12 years	pT2NXMX	Ovarian	–	CR/LFU	3	2	3	2	3	1.04	+
KT 89/87P[a]	17 years	pT1NXMX	Ovarian	–	CR/LFU	3	6	NA	3	3	1.50	+
KT 1076/91P[a]	6 years	pT1NXMX	Testicular	–	?	4	4	NA	2	4	1.94	+
KT 774/91P[a]	3 years	pT1NXMX	Testicular	–	?	3	4	4	2	3	1.41	+
KT 169/86P[a]	1 years	pT1NXMX	Testicular	–	?	4	4	NA	2	3	1.54	NA
KT 429/90P[a]	1.5 years	pT1NXMX	Testicular	–	?	2	8	4	2	4	1.96	NA
KT 453/90Me[a]	3 years	pTXNXM1	Testicular	Renal pelvis	?	4	4	4	NA	3	1.63	–
Control[a]	F					NA	2	NA	2	NA	0.9–1.10	–
	M					2	2	2	1	2	0.9–1.10	–

[a] The ISH results for gonadal yolk sac tumours and controls published by Jenderny et al. (1995)

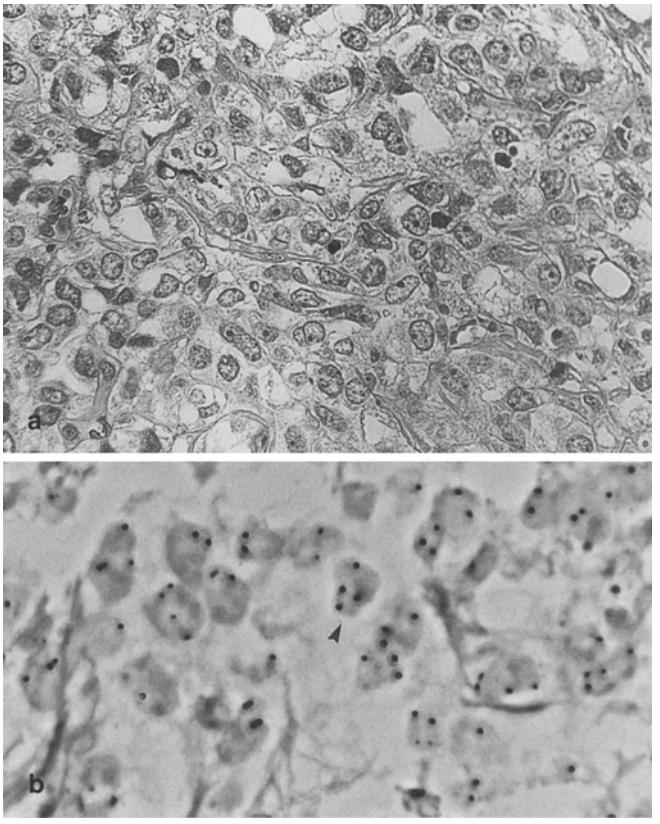

Fig. 1 **a** Morphological appearance of the paediatric pure yolk sac tumour KT 379/90 using hematoxylin-eosin counterstain (original magnification, × 125). **b** In situ hybridization ISH signals in interphase nuclei of a paraffin section (5 µm) using indirect peroxidase with diaminobenzidine staining and haematoxylin-eosin counterstain (original magnification, × 280). Nucleus (*arrowhead*) from the paediatric pure yolk sac tumour area KT 379/90 was tetrasomic for chromosome 1 after ISH with the pericentromere-specific DNA probe D1Z1

Usually the DIs correlated well with the ISH findings of chromosome 8 (Table 2).

Discussion

Very few karyotypic analyses have been performed on extragonadal YSTs of childhood. The investigation of one extragonadal, radiation-induced paediatric pure YST of a 35-year-old man revealed a "pseudo" diploid karyotype with translocations and a monosomy of 12p and 7q [1]. Three extragonadal pure YSTs and one mixed GCT with a YST component involving children under 3 years of age were in the diploid or tetraploid range and showed structural aberrations involving chromosomes 1, 3, and 6. Moreover, the occurrence of a deletion in the short arm of chromosome 1 in all extragonadal pure YSTs raised the question if this aberration is an important event in this paediatric histological subtype [9].

In the present study, most paediatric extragonadal pure YSTs and the YST component had similar aberrations to GCTs of adults. They also had a high incidence of supernumerary chromosomes. Aberrations in chromosome number were usually found to be correlated with changes in DNA content, so that the ISH results reflected the ploidy grade of the tumours. For example, in tumour KT 379/90 up to four hybridization signals were observed with the DNA probes of chromosomes 1, 8, 12, 17, and X, respectively. This corresponded well with a DI of 1.92. The low frequency of nuclei with the maximum of hybridization signals is mainly due to truncated nuclei, but the occurrence for example of diploid or triploid cells within a predominantly tetraploid tumour population cannot be excluded. The tumour KT 442/89 was characterized by a histogram with a stemline in the trip-

loid range (DI: 1.45). When compared with the DNA cytometry analysis, the ISH study on paraffin sections detected tumour cell clones with up to four hybridization signals with the DNA probes of chromosomes 1, 8, 12, and X, respectively. In this tumour, the relatively low percentages of nuclei with the highest copy number may reflect a small subpopulation of tetraploid cells within a predominantly triploid tumour cell population. Both examples illustrated that a heterogeneous pattern in chromosome copy numbers obtained from ISH on truncated nuclei from paraffin sections can cause problems in the interpretation of the actual copy number of the target chromosomes. Therefore, for the exact assessment of the copy number of chromosomes in tumour cells, ISH on isolated (intact) nuclei may provide a better target for the examination of a mixed tumour cell population than paraffin sections. However, as discussed briefly by Stock et al. [12], the histological context is completely lost when isolated nuclei are analysed and normal cells cannot be distinguished from disomic tumour cells of equal size. Furthermore, the advantage of ISH on paraffin sections is the exclusion of non-tumour cells. Only one paediatric extragonadal pure YST KT 15/86 failed to show numerical aberrations with the DNA probes used. Since a DI of 1.23 was present, it is possible that this tumour is affected by other numerical chromosome aberrations. The three other paediatric extragonadal pure YSTs and the YST component were characterized by gains of one or two additional copies of chromosomes 12 followed by gains of chromosome 1, and/or 8, 17, X. Recently, it has been possible to analyse three paediatric extragonadal pure YSTs or mixed GCTs with a YST component in their histological context on paraffin sections by ISH with similar chromosome-specific DNA probes and by flow cytometry [12]. In accordance with our study these tumours showed an over-representation of chromosomes 1, 8, 10, 12, and X. The DI values of the tumours (1.6, 1/1.91 and 1.2/2.4) correspond with our results.

A specific structural chromosome aberration, an isochromosome i(12p) of the short arm of chromosome 12 was detected in adult GCTs in nearly all histologies and sites [10, 11]. Similar to GCTs of adult, paediatric GCTs were characterized by the gain of one or more chromosomes 12. Whether an over-representation of chromosomes 12 represented the specific marker (i12p) cannot be detected with this method. However, Mukherjee et al. [8] reported the rapid detection of the i(12p) marker chromosome in interphase nuclei of adult testicular GCTs. Using the DNA probe D12Z3 the hybridization signals of an i(12p) can be distinguished from those of a normal chromosome 12 by their larger or smaller sizes. However, we did not see this phenomenon in any of the extragonadal GCTs we investigated on paraffin sections. On the other hand, in two gonadal pure YSTs we recognized smaller and larger hybridization signals after ISH with the DNA probe D12Z3. These size differences were restricted to the tumour areas, the normal or stromal cells of both tumours showed normal hybridization signals of chromosome 12. Unfortunately, we could not analyse isolated nuclei from both tumours and/or interphase cells from a documented i(12p)-positive GCT [6]. Interestingly, Stock et al. [12, 13] detected smaller D12Z3 hybridization signals than those of normal chromosomes 12 in isolated nuclei of one extragonadal mixed GCT with a YST component.

Like the GCTs of adults, the short arm of chromosome 1 was often involved in structural aberrations. One paediatric extragonadal pure YST and one YST component showed 1p36.3 deletions after ISH with the DNA probe D1S32. Stock et al. [12] detected similar deletions with an analogous DNA probe in all three analysed paediatric extragonadal pure YSTs or mixed GCTs with a YST component. Moreover, three further ISH reports on paediatric gonadal and extragonadal GCTs showed 1p36.3 deletions in dysgerminoma, seminoma, embryonal carcinoma, YSTs, and choriocarcinoma [6, 12, 13]. Interestingly, Stock et al. [13] did not observe 1p36.3 deletions in paediatric mature and immature teratomas. In agreement with Stock et al. [12, 13], we observed 1p36.3 deletions in early and advanced tumour stages (present data; [6]). The identification of 1p36.3 deletions in a large series of paediatric GCTs suggests that this abnormality may play a role in the pathogenesis of nearly all types of paediatric GCT at all sites – with the possible exception of mature and immature teratomas.

Acknowledgements This work was supported by the Deutscher Akademischer Austauschdienst and Schleswig-Holsteinische Krebsgesellschaft. The DNA probes D1Z1 and D1S32 were kindly provided by P.F. Ambros. We thank K. Dege for her help in editing the manuscript.

References

1. Cowan JM, Beckett MA, Tarbell NJ, Weichselbaum RR (1990) Monosomy 12p in a radiation-induced germ cell tumour. Genes Chromosom Cancer 2: 186–190
2. Harms D, Jänig U (1986) Germ cell tumours of childhood. Virchows Arch [A] 409: 223–239
3. Hawkins EP (1990) Pathology of germ cell tumours in children. Oncol Hematol 10: 165–179
4. Hedley DW, Friedlander ML, Taylor IW, Rugg CA, Musgrove EA (1983) Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. J Histochem Cytochem 31: 1333–1335
5. Hopman AHN, Van-Hooren E, Van de Kaa CA, Vooijs PG, Ramaekers FCS (1991) Detection of numerical aberrations using in situ hybridization in paraffin sections of routinely processed bladder cancers. Mod Pathol 4: 503–513
6. Jenderny J, Köster E, Meyer A, Borchers O, Grote W, Harms D, Jänig U (1995) Detection of chromosome aberrations on paraffin sections of seven gonadal yolk sac tumours of childhood. Hum Genet 96: 644–650
7. Maddox PH, Jenkins D (1987) 3-Aminopropyl triethoxysilane (Apes): a new advance in section adhesion. J Clin Pathol 40: 1256–1260
8. Mukherjee AB, Murty VVVS, Rodriguez E, Reuter VE, Bosl GJ, Chaganti RSK (1991) Detection and analysis of origin of i(12p), a diagnostic marker of human germ cell tumours, by fluorescence in situ hybridization. Genes Chromosom Cancer 3: 300–307

9. Perlman EJ, Cushing B, Hawkins E, Griffin CA (1994) Cytogenetic analysis of childhood endodermal sinus tumours: a paediatric oncology group study. Pediatr Pathol 14: 695–708
10. Rodriguez E, Mathew S, Reuter V, Ilson DH, Bosl GJ, Chaganti RSK (1992) Cytogenetic analysis of 124 prospectively ascertained male germ tumours. Cancer Res 52: 2285–2291
11. Samaniego F, Rodriguez E, Houldsworth J, Murty VVVS, Ladanyi M, Lele KP, Chen Q, Dmitrovsky E, Geller NL, Reuter V, Jhanwar SC, Bosl GJ, Chaganti RSK (1990) Cytogenetic and molecular analysis of human male germ cell tumours: chromosome 12 abnormalities and gene amplification. Genes Chromosom Cancer 1: 289–300
12. Stock C, Ambros IM, Lion T, Haas OA, Zoubek A, Gadner H, Ambros PF (1994) Detection of numerical and structural chromosome abnormalities in paediatric germ cell tumours by means of interphase cytogenetics. Genes Chromosom Cancer 11: 40–50
13. Stock C, Ambros IM, Strehl S, Zoubek A, Fink FM, Gadner H, Ambros PF (1995) Zytogenetische Aspekte pädiatrischer Keimzelltumouren. Klin Pädiatr 207: 235–241