Virchows Arch (1999) 434:11–15 © Springer-Verlag 1999

ORIGINAL ARTICLE

H. Herbst · C. Kühler-Obbarius · H. Lauke
M. Sauter · N. Mueller-Lantzsch · D. Harms
T. Löning

Human endogenous retrovirus (HERV)-K transcripts in gonadoblastomas and gonadoblastoma-derived germ cell tumours

Received: 26 May 1998 / Accepted: 28 July 1998

Abstract Gonadoblastomas are rare tumours of abnormal or dysgenetic gonads, often transforming to invasive seminomatous and nonseminomatous germ cell tumours (GCT). Because of the intimate association of noninvasive and invasive lesions, gonadoblastoma may provide clues as to the molecular pathogenesis of GCT. We studied the expression of the human endogenous retrovirus (HERV)-K *gag* gene in eight gonadoblastomas arising in phenotypically female patients, including two newborn girls. We also studied testicular biopsies with immature Sertoli cell nodules harbouring neoplastic germ cells, a lesion with morphological resemblance to gonadoblastoma. In five gonadoblastomas, invasive seminoma/dysgerminoma was noted, in two cases with formation of additional GCT components. HERV-K *gag* transcripts were found with moderate levels in gonocytes of all gonadoblastomas and in neoplastic germ cells in testicular Sertoli cell nodules. All invasive GCT except for teratomas displayed HERV-K transcripts. Thus, expression of HERV-K is induced during fetal or embryonal development and precedes invasive GCT formation. Although the specific role of HERV-K expression remains unknown, the findings place HERV-K expression in an appropriate time frame for it to have a role in the molecular pathogenesis of GCT and suggest a precursor-invasive tumour relationship for ovarian GCT equivalent to the more common carcinoma in situ of the testis and testicular GCT.

Key words Gonadoblastoma · Germ cell tumour · Human endogenous retrovirus

H. Herbst (✉)
Institut für Pathologie, Universitätskrankenhaus Eppendorf, Martinistrasse 52, D-20246 Hamburg, Germany
e-mail: herbst@uke.uni-hamburg.de
Tel.: +49-40-4717 2859, Fax: +49-40-4717 4961

C. Kühler-Obbarius · T. Löning
Abteilung für Gynäkopathologie, Universitätskrankenhaus Eppendorf, Hamburg, Germany

H. Lauke
Institut für Anatomie, Universitätskrankenhaus Eppendorf, Hamburg, Germany

M. Sauter · N. Mueller-Lantzsch
Abteilung Virologie, Universitätskliniken des Saarlands, Homburg/Saar, Germany

D. Harms
Institut für Paidopathologie der Universität, Kiel, Germany

Introduction

Gonadoblastomas are rare neoplasms composed of an intimate admixture of gonocytes and sex cord/gonadal stromal elements, resembling immature Sertoli and granulosa cells and, less frequently, Leydig cells [14, 15]. Virtually all gonadoblastomas arise in dysgenetic gonads. Rare examples have been found in otherwise normal ovaries or descended testes. Gonadoblastomas are therefore thought to reflect a disorderly and abortive attempt to form a gonad in a genetically abnormal individual [14, 15]. About half the reported cases showed invasive growth of the germ cell component, usually resulting in a pattern of seminoma/dysgerminoma. In a small proportion of cases, however, the invasive tumour component also displayed nonseminomatous germ cell tumour (GCT) morphology. Approximately 10% of the cases with invasive tumour growth also demonstrated metastatic spread [15, 20].

The gonocytes present in gonadoblastomas display morphological and immunophenotypic similarities with cells of the so-called carcinoma in situ of the testis (testicular intraepithelial neoplasia, CIS/TIN) [2, 3, 7, 17, 18]. This relates in particular to the high content of glycogen resulting in a clear cytoplasm and expression of the germ cell-specific isoform of alkaline phosphatase. Because of these characteristics gonadoblastoma can be regarded as a model for the study of the relationship between noninvasive neoplastic germ cells and invasive GCT [7, 15], as postulated for CIS/TIN and testicular GCT [2, 3, 17, 18].

Recent research has provided good morphological evidence for a link between testicular and ovarian GCT and gestational chorionic carcinoma, with expression of a distinct family of human endogenous retrovirus (HERV) sequences, HERV-K. Elevated titres of HERV-K Gag (group-specific antigen) and Env (envelope) protein-specific antibodies were found in patients with seminomas and some other testicular GCT [12, 13]. Using nonoverlapping probes specific for the *gag*, *prt*, *pol*, and *env* genes of the prototype proviral sequence, HERV-K10, high levels of corresponding RNA transcripts were observed in tumour cells of testicular and ovarian GCT as well as in cases of gestational chorionic carcinoma [5]. With the exception of teratoma (as defined by the WHO) and spermatocytic seminoma, all GCT entities and CIS/TIN, the presumed precursor of all testicular GCT except spermatocytic seminoma [18], consistently expressed HERV-K sequences [5]. Furthermore, detection of HERV-K Gag protein by Western blot indicated that the RNA transcripts are indeed translated in the tumour cells [5, 12].

Using in situ hybridization with [^{35}S]-labelled HERV-K10 *gag* probes, we have extended these studies to a series of gonadoblastomas arising in abnormal ovaries or streak gonads of phenotypically female patients, some of which displayed the presence of dysgerminoma or non-dysgerminomatous GCT. Additionally, we studied hypoplastic testicular areas (or immature Sertoli cell nodules) [4, 6, 19], lesions bearing a morphological resemblance to gonadoblastoma. We show that HERV-K transcripts are already present in gonocytes occurring in gonadoblastoma and immature Sertoli cell nodules and are expressed at increased levels in the evolving invasive GCT.

Materials and methods

Archival tissues had been fixed in neutral-buffered formalin and embedded in paraffin wax. Sections (5 µm) were cut and mounted onto glass slides pretreated with 3-aminopropyl-triethoxysilane (APES). Histological diagnosis followed the criteria set out by the World Health Organization (WHO) for testicular and ovarian tumours [10, 16]. Twelve cases of seminoma and 4 testicular biopsies with CIS/TIN served as controls. In 2 seminomas, testicular tissue adjacent to the invasive tumour contained immature Sertoli cell nodules associated with atypical germ cells and occasional areas of calcification resembling gonadoblastoma. Further, tissue blocks were available from 8 cases of gonadoblastoma arising in phenotypic females.

Case 1: A 15-year-old girl with bilateral gonadoblastoma, bilateral dysgerminoma and unilateral teratoma with mucinous differentiation.

Case 2. A 19-year-old patient with unilateral gonadoblastoma displaying extensive calcification and focal development of dysgerminoma.

Case 3. A 13-year-old girl with a unilateral tumour in the right ovary comprising a small nest of gonadoblastoma and larger areas of mixed GCT including embryonal carcinoma, yolk sac tumour, chorionic carcinoma and immature teratoma; the contralateral ovary was hypoplastic and free of tumour.

Case 4. A 10-day-old girl with a unilateral indifferent ovary displaying small foci of gonadoblastoma.

Case 5. Aa 18-year-old patient with Turner's syndrome and a unilateral indifferent gonad harbouring a small, extensively calcified gonadoblastoma.

Case 6. A 25-day-old girl with a prenatally diagnosed ovarian cyst showing extensive haemorrhagic infarction and small nests of gonadoblastoma in association with some luteinized cells.

Case 7. A 7-year-old girl displaying small nests of gonadoblastoma in an otherwise normal right ovary largely destroyed by dysgerminoma and a normal left ovary.

Case 8. An 11-year-old patient with Turner's syndrome and bilateral gonadal dysgenesis, the left gonad displaying multiple small nests of gonadoblastoma largely overgrown by dysgerminoma.

The embryonal carcinoma cell line, Tera-1, was obtained from the American Type Culture Collection, Rockville, Md. (ATCC #HTB105) [1]. The B95-8 cell line was originally established by infecting marmoset B lymphocytes with Epstein-Barr virus. Formalin-fixed and paraffin-embedded cell pellets were sectioned like tissues to serve as positive control.

Single-stranded nonoverlapping probes specific for the HERV-K10 *gag* sequences were prepared as recently described [5]. After linearization of the plasmids with either *Hin*dIII or *Eco*RI restriction endonucleases, T7 or SP6 RNA-polymerases (BRL Gibco, Eggenstein, Germany), respectively, were used to obtain run-off transcripts of either the anti-sense (complementary to mRNA), or sense (anti-complementary, negative control) strands in the presence of [^{35}S]ribonucleotides [5].

Following hybridization and washing, bound probes were detected by autoradiography as previously described [5].

Results

HERV-K-specific transcripts were detectable in cell pellets prepared from Tera-1 cells, but were absent from the EBV-positive cell line B95-8. In all gonadoblastomas, proportions of gonocytes displayed HERV-K transcripts at low levels requiring an average of 12 days of autoradiographic exposure (Fig. 1a, b). However, only parts of the tumours could be evaluated confidently, because of autoradiographic artefacts caused by extensive intralesional sclerosis and

Table 1 HERV expression in components of gonadoblastoma and evolving GCT (– no specific labelling compared with sense control hybridizations, + to ++++ autoradiographic labelling indicating presence of HERV gag transcripts with variable levels after 12 days of autoradiography, specifically: + weak labelling, few silver grains were noted over the labelled cells, ++ moderate labelling leaving nuclear structures visible, +++ strong labelling concealing the nuclei, ++++ very strong labelling concealing nuclei and cytoplasm)

Case no.	1	2	3	4	5	6	7	8
Gonocytes	+	+	+ to ++	+	+	+	+	+
Stromal cells	–	–	–	–	–	–	–	–
Sex cord cells	–	–	–	–	–	–	–	–
Dysgerminoma	++	++	+++				++	++
Embryonal carcinoma			++++					
Yolk sac tumour			++					
Choriocarcinoma			+++					
Teratoma	–		–					

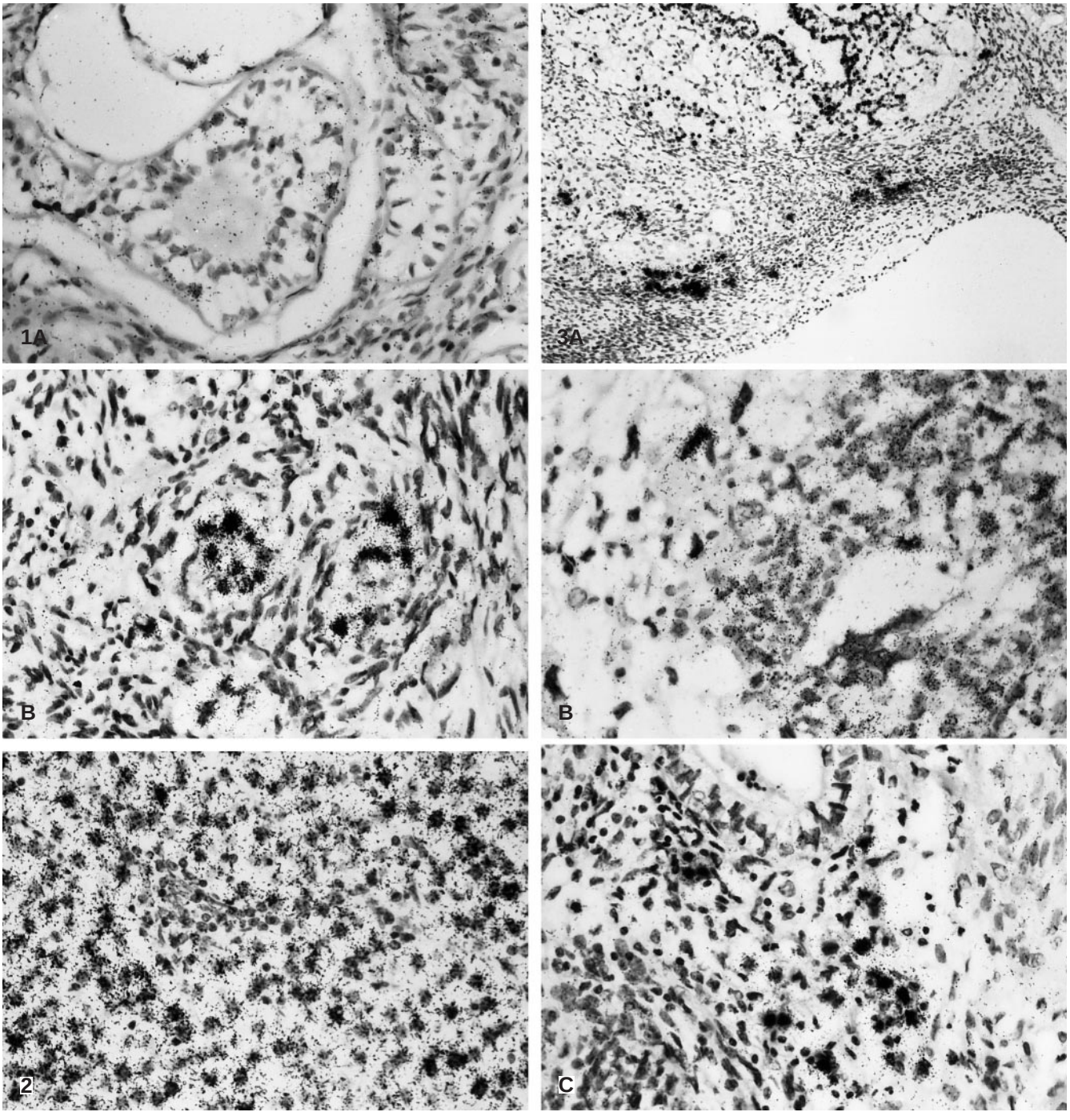

Fig. 1 Detection of HERV-K *gag* transcripts in two cases of gonadoblastoma (cases 3, 4), one of which was partially overgrown by a mixed germ cell tumor. Tumour cells display a variable autoradiographic signal which is absent from lymphocytes, stromal and sex cord cells. Paraffin section, autoradiographic exposure 8 days, original magnification ×110

Fig. 2 HERV-K gag RNA expression in a gonadoblastoma-associated dysgerminoma carcinoma (case 7). A strong autoradiographic signal is restricted to tumour cells and absent from neighbouring areas. Paraffin section, autoradiographic exposure 8 days, original magnification ×90

Fig. 3 Gonadoblastoma-associated mixed germ cell tumor (case 3) with HERV-K *gag* RNA expression in tumour components such as **A** yolk sac tumour, **B** choriocarcinoma, and **B**, **C** scattered dysgerminoma cells except for **C** teratomatous areas. A strong autoradiographic signal is restricted to tumour cells and absent from neighbouring stroma. Paraffin section, autoradiographic exposure 8 days, original magnification **A** ×65, **B**, **C** ×105

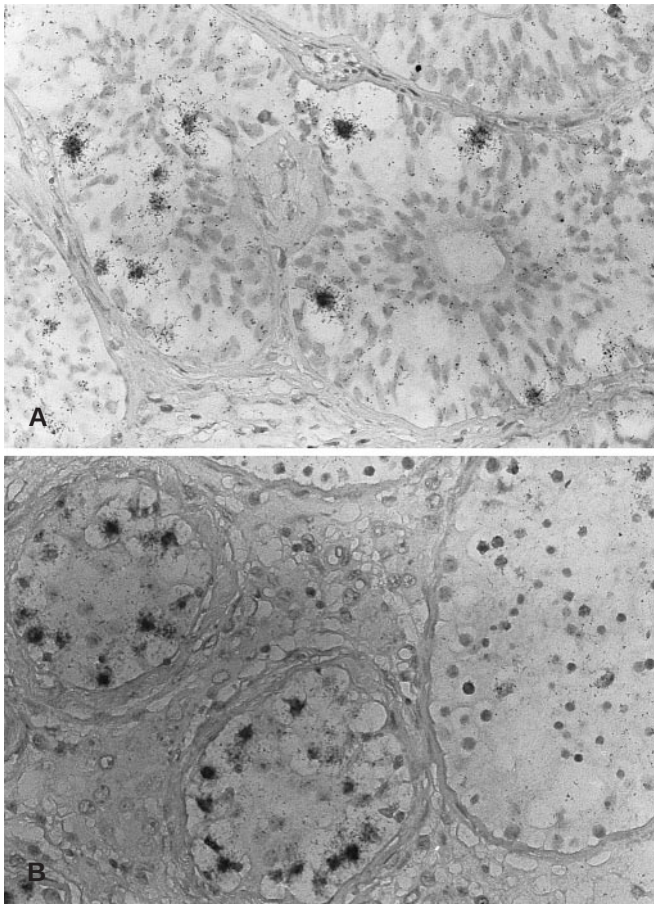

Fig. 4 HERV-K gag RNA expression by testicular CIS/TIN (carcinoma in situ or testicular intraepithelial neoplasia) cells **A** in an immature Sertoli cell nodule and **B** in adjacent normally formed tubuli. Paraffin section, autoradiographic exposure 12 days, original magnification ×135

calcification in some cases. In 5 gonadoblastomas, the tumour was partially overgrown by invasive seminoma/dysgerminoma (Fig. 2), and in 2 of these cases by additional GCT components including embryonal carcinoma, yolk sac tumour, chorionic carcinoma and teratoma (Fig. 3a–c; Table 1). With the exception of teratomatous elements, all GCT components displayed HERV-K transcript levels above those observed in the atypical gonocytes present in the gonadoblastoma component. Comparing the various tumour components in individual sections of case 3, for example, the most intense labelling was noted over embryonal carcinoma cells (Table 1). In the seminoma cases included as positive control tissues, seminoma and CIS/TIN cells displayed HERV *gag* transcripts as previously described [5, 12], whereas only background labelling was found in cells in normal spermatogenesis and in the other tissue components. In Sertoli cell nodules, neoplastic gonocytes equivalent to CIS/TIN cells displaying HERV-K transcripts at high levels were present both in the immature testicular areas and in normally formed adjacent tubules, whereas Sertoli cells did not show autoradiographic signals above background levels (Fig. 4a, b).

Discussion

Gonadoblastomas bear a high risk of transformation to invasive GCT. In cases that have not been entirely overgrown by a GCT, the intralesional relationship between the malignant GCT and its precursor cells, neoplastic germ cells, is morphologically irrefutable [15]. Atypical gonocytes of gonadoblastoma and testicular CIS/TIN share numerous phenotypic properties and display a similar degree of intralesional heterogeneity [7]. In our series of gonadoblastomas arising in abnormal ovaries and dysgenetic gonads, we found consistent expression of HERV-K at the transcriptional level in neoplastic germ cells and in tumour cells of all invasive GCT components except for teratoma. In addition to the direct intralesional association of all tumour components, this provides a molecular phenotypic correlate for the role of neoplastic gonocytes as precursors of invasive ovarian GCT. The patterns of HERV-K expression in gonadoblastoma-associated GCT are identical to those previously established for malignant testicular and ovarian GCT arising in mature, fully differentiated gonads and suggest a common molecular pathogenesis of most GCT entities. The finding of HERV-K expression in ovaries of newborn girls also supports the hypothesis that GCT precursors are formed during embryonic or fetal development in utero. Based on the observation of HERV-K expression in gonocytes of dysgenetic gonads and gonadoblastoma and morphologically diverse forms of GCT, and by analogy with CIS/TIN and testicular GCT, pre-invasive HERV-K-expressing gonocytes may be postulated as precursors for invasive GCT in the mature ovary. In most cases, though, it may be virtually impossible to identify those precursor cells because, at the time of diagnosis, they are likely to be overgrown by the emerging malignancy [15, 20].

Immmature Sertoli cell nodules or hypoplastic areas represent testicular lesions with morphological similarities to ovarian gonadoblastoma, with respect to both the sex cord cell component and, if present, the neoplastic germ cells. These nodules are often seen in the vicinity of invasive GCT, provided that uninvolved testicular tissue is adequately sampled [4, 6, 19]. The finding of HERV-K expression in atypical gonocytes in immature Sertoli cell nodules provides a link between these lesions and invasive GCT.

Testicular GCT are often associated with CIS/TIN occurring in the immediate vicinity of the invasive tumour. This finding has been interpreted as intratubular spread of seminoma cells (“intratubular seminoma”) or, alternatively, considered to be a remainder of the intratubular “in situ” precursor lesion of the invasive GCT. The finding of immature Sertoli cell nodules populated by HERV-K-positive CIS/TIN cells in GCT-bearing testes lends credence to the notion that GCT are often associated with developmental lesions. Arguments for the latter view are also provided by the increased frequency of CIS/TIN contralateral to the tumour-bearing testicle and by its elevated incidence in maldescended testes, a condition associated with an increased risk of developing overt GCT [2, 3, 17, 18].

As previously observed, teratomas, regardless of their degree of maturity, did not display autoradiographic signals specific for HERV-K RNA. Therefore, these tumours cannot be traced back with confidence to a particular subpopulation of gonocytes. Because of the intimate association of HERV-K-negative teratomatous and HERV-K-positive non-teratomatous invasive GCT components arising within a gonadoblastoma (case 3), it is most likely that HERV-K-expressing atypical gonocytes are precursors for both tumour components. This implies that HERV-K expression is down-regulated once differentiation towards an embryonic tissue is initiated. The observation of HERV-K-positive CIS/TIN cells adjacent to pure testicular teratomas also supports this view [5]. In contrast to testicular teratomas, however, the molecular aetiology of ovarian teratomas seems to be variable. Most ovarian teratomas are clinically benign, diploid tumours without isochromosome i(12p) formation [11] and are therefore unlikely to derive from an ovarian homologue to testicular CIS/TIN.

The study further confirms a tight association between HERV-K gene expression and invasive GCT and suggests the existence of testicular and ovarian precursor cells for these tumours that may already be present at birth. The function of retroviral gene products and their biological significance, however, are not clear at present. Because in situ hybridization does not discriminate spliced from unprocessed transcripts, it remains to be seen whether the RNA detected by in situ hybridization represents processed transcripts. However, the splicing patterns of HERV-K transcripts and particle formation in teratocarcinoma cell lines [8, 9], and the detection of HERV-K Gag- and Env-specific antibodies in sera from GCT patients [2, 18] together with the demonstration of 73-kDa Gag polyprotein in GCT protein extracts by Western blotting [5, 12] support the notion that HERV-K gene expression in GCT is not restricted to the level of transcription, but results in translation and processing of proteins.

It will be of particular interest to extend the studies to nonstructural proteins potentially encoded by HERV-K genomes. Spliced mRNA homologous to the Rev protein of the human immunodeficiency virus HIV-1 was recently demonstrated in teratocarcinoma cell lines [9]. Such proteins may have functions as transcription factors and may influence growth and phenotypic properties of HERV-K-expressing cells. The variation in HERV-K transcript levels in malignant germ cells may then indicate a cell-cycle-dependent control of HERV-K expression and a relationship to proliferation. It is not yet clear whether certain HERV-K gene products may confer a proliferative advantage, thus representing a first step in the chain of events ultimately leading to invasive germ cell tumours.

Acknowledgements The authors are grateful to Mrs K. von Ostau for her expert technical assistance and to Mr H.J. Koppelmeyer for help with the photos. The work was supported by the Deutsche Forschungsgemeinschaft, the Werner-Otto-Stiftung, and the Hamburger Stiftung zur Förderung der Krebsbekämpfung.

References

1. Bronson DL, Fraley EE, Fogh J, Kalter SS (1979) Induction of retrovirus particles in human testicular tumour (Tera 1) cell cultures: an electron microscopic study. J Natl Cancer Inst 63: 337–339
2. Dieckmann KP, Loy V (1997) Prevalence of contralateral testicular intraepithelial neoplasia in patients with testicular germ cell neoplasia. J Clin Oncol 14:3126–3132
3. Dieckmann KP, Loy V, Huland H (1989) Carcinoma in situ of the testis: clinical significance, diagnosis and therapy. Urologe [A] 28:271–280
4. Hedinger CE, Huber R, Weber E (1967) Frequency of so-called hypoplastic or dysgenetic zones in scrotal and otherwise normal human testes. Virchows Arch [A] 342:165–168
5. Herbst H, Sauter M, Mueller-Lantzsch N (1996) Expression of human endogeneous retrovirus K elements in germ cell and trophoblastic tumours. Am J Pathol 149:1727–1735
6. Holstein AF, Schütte B, Becker H, Hartmann M (1987) Morphology of normal and malignant germ cells. Int J Androl 10: 1–18
7. Jorgensen N, Muller J, Jaubert F, Clausen OP, Skakkebaek NE (1997) Heterogeneity of gonadoblastoma germ cells: similarities with immature germ cells, spermatogonia and testicular carcinoma in situ cells. Histopathology 30:177–186
8. Löwer R, Boller K, Hasenmaier B, Korbmacher C, Mueller-Lantzsch N, Löwer J, Kurth R (1993) Identification of human endogeneous retroviruses with complex mRNA expression and particle formation. Proc Natl Acad Sci USA 90: 4480–4484
9. Löwer R, Tönjes, RR, Korbmacher C, Kurth R, Löwer J (1995) Identification of a rev-related protein by analysis of spliced transcripts of the human endogeneous retrovirus HTDV/HERV-K. J Virol 96:141–149
10. Mostofi FK, Sesterhenn IA (1998) Histological typing of testis tumours. International histological classification of tumours, 2nd edn. Springer, Berlin Heidelberg New York
11. Oosterhuis JW, Castedo SMMJ, de Jong B (1990) Cytogenetics, ploidy and differentiation of human testicular, ovarian and extragonadal germ cell tumours. Cancer Surv 9:321–332
12. Sauter M, Schommer S, Kremmer E, Remberger K, Dölken G, Lemm I, Buck M, Best B, Neumann-Haefelin D, Mueller-Lantzsch N (1995) Human endogenous retrovirus HERV-K: expression of gag protein and detection of antibodies in patients with seminomas. J Virol 69:414–421
13. Sauter M, Roemer K, Best B, Afting M, Schommer S, Seitz G, Hartmann M, Mueller-Lantzsch N (1996) Specificity of antibodies directed against Env proteins of human endogenous retroviruses in patients with germ cell tumours. Cancer Res 56:444362–444365
14. Scully RE (1963) Gonadoblastoma. A gonadal tumor related to dysgerminoma (seminoma) and capable of sex hormone production. Cancer 6:455–463
15. Scully RE (1970) Gonadoblastoma. Cancer 25:1340–1356
16. Serov SF, Scully RE, Sobin LH (1973) Histological typing of ovarian tumours. International histological classification of tumours No 9. World Health Organization, Geneva
17. Skakkebaek NE (1972) Possible carcinoma-in-situ of the testis. Lancet II:516–517
18. Skakkebaek NE, Berthelsen JG, 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
19. Sohval AR (1956) Testicular dysgenesis in relation to neoplasm of the testicle. J Urol 75:285–291
20. Talerman A (1974) Gonadobastoma associated with embryonal carcinoma. Obstet Gynecol 43:138–142