Glycolipids of human primary testicular germ cell tumours

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Summary The glycolipid content of human non-seminomatous germ cell tumour cell lines correlates with their differentiation lineage. To analyse whether this reflects the situation in primary tumours, we studied five embryonal carcinomas, five yolk sac tumours and nine (mixed) non-seminomas, using thin-layer chromatography and carbohydrate immunostaining. We also analysed the glycolipid content of 19 seminomas to reveal their relationship with non-seminomas. Lactosylceramide (CDH) was detected in all embryonal carcinomas, but in fewer than half of the seminomas. Seminomas and embryonal carcinomas contained globoseries glycolipids, including globotriosylceramide (Gb3), globoside (Gb4), galactosyl globoside (Gb5) and sialyl galactosyl globoside (GL7). The lacto-series glycolipid Le4 was found in all embryonal carcinomas, but only in one seminoma. Gangliosides GD3 and GT3 were detected in many seminomas, but rarely in embryonal carcinomas. Yolk sac tumours displayed a heterogeneous glycolipid profile. Compared with seminomas and pure embryonal carcinomas, differentiated non-seminomas had reduced levels of globoseries glycolipids, especially Gb3 and Gb5, whereas CDH, Le4, GD3 and GT3 were found in the majority of cases. Thus, the glycolipid content of non-seminoma cell lines reflects the situation in primary tumours. Globoseries glycolipids are similarly expressed in seminomas and embryonal carcinomas. The expression of Gb3 and Gb5 is reduced in non-seminomas upon differentiation. Le4 expression, allowing discrimination from seminomas, was shown to increase in yolk sac tumours. Expression of gangliosides in seminomas might indicate their maturation from yolk sac cell precursors. Reprogramming of these precursor cells would result in the formation of Le4-expressing embryonal carcinomas.

Keywords: glycolipid; human primary testicular germ cell tumour; differentiation; pathogenetic relation

In humans, two entities of testicular germ cell tumours (TGCTs) of adolescents and adults can be distinguished: seminomas (SEs), which are composed of tumour cells that are considered to be the malignant counterpart of human primordial germ cells, and non-seminomatous TGCTs (NSs), comprising embryonal carcinoma (EC), the undifferentiated stem cells of human NSs), immature and mature teratoma (IT and MT), yolk sac tumour (YS) and choriocarcinoma (CC) (Mostofi et al., 1987). The relationship between SEs and NSs is a matter of debate. Several investigators suggest that SE and NS are biologically independent (Pierce and Abell, 1970; Mostofi, 1984; Sesterhenn, 1985), whereas others assume that NS develops through a, not necessarily clinically manifest, SE stage (Friedman, 1951; Oliver, 1987; Oosterhuis et al., 1989; Oosterhuis and Looijenga, 1993). According to this so-called linear progression model, SE cells become ‘reprogrammed’ to EC cells. This hypothesis is supported by morphological, ultrastructural, immunohistochemical (interphase), chromosomal and clinical analyses (Oosterhuis et al., 1989; De Jong et al., 1990; Oliver, 1990; Czernobilsky, 1991; Fossè et al., 1991; Czaja and Ulbright, 1992; El-Naggar et al., 1992; Looijenga et al., 1993).

The study of human NSs is facilitated by the existence of cell lines representing most non-seminomatous cell types (Pattillo et al., 1971; Fogh and Trempe, 1975; Andrews et al., 1980; Oosterhuis et al., 1985; Casper et al., 1987; Pera et al., 1987; Damjanov et al., 1993; von Keitz et al., 1995). Experiments can be performed using cell lines of pluripotent EC cells, which can be induced to differentiate by exposure to certain agents (e.g. retinoic acid and hexamethylene bisacetamide), for example allowing analysis of changes in gene expression responsible for, or coinciding with, the process of differentiation. Some of these studies have focused on the expression of cell-surface glycolipids, i.e. molecules composed of a carbohydrate and a lipid moiety. Various groups of glycolipids can be distinguished according to their basic molecular structure (IUPAC-IUB, 1978). The three main groups are the so-called globo-, lacto-, and ganglio-series glycolipids (Svenssmoïlholm, 1964). Among others, glycolipids are involved in early embryonic development and in mediation/modification of growth factor action (Bird and Kimber, 1984; Bremer et al., 1984; Fenderson et al., 1984; Cuello et al., 1989; Eggens et al., 1989). Therefore, glycolipids might be important in the development of TGCTs. The patterns of glycolipid expression in non-seminomatous cell lines correlate with their differentiation lineage. Andrews et al. (1990) and Wenk et al. (1994) have shown that EC cell lines are characterised by the expression of globo-series glycolipids, including globotriosylceramide (Gb3), globoside (Gb4), galactosylgloboside (Gb5) and sialyl galactosylgloboside (GL7). Upon induced or spontaneous differentiation of these cells into the various non-seminomatous cell types the synthesis of globo-series glycolipids is down-regulated, whereas the synthesis of lacto- and ganglio-series glycolipids (including Le4, and GD3/GT3 respectively) increases. Cell lines derived from YSs can contain Gb3, Gb4 and gangiosides, whereas EC cell lines mainly express Gb3 and the stage-specific embryonic antigen 1 (SSEA-1)-carrying lacto-series glycolipid Le4 (Wenk et al., 1994). Thus, specific combinations of glycolipids are correlated with specific cell types and the way in which the various cell types are related can be studied using glycolipid analysis. No extensive data on the glycolipid pattern of primary SEs and NSs have been reported (Ohyama et al., 1990, 1992), we therefore studied the glycolipids of carcinoma in situ (CIS, the precursor of all TGCTs; Skakkebaek et al., 1987) and primary TGCTs to reveal the relationship between SEs and NSs, especially ECs.

Materials and methods

Tumour handling

Forty-nine orchidectomy specimens, suspected of a germ cell tumour were collected in the operation theatre or pathology department of collaborating hospitals. Representative parts
of tumour and adjacent normal parenchyma were snap frozen using liquid nitrogen. The remaining parts were put in culture medium [Dulbecco's modified Eagle medium (DMEM)F12, with 103 KU l⁻¹ penicillin, 103 mg l⁻¹ streptomycin, 43 mg l⁻¹ gentamycin, 365 mg l⁻¹ glutamin, Gibco, Paisley, UK] and taken to the laboratory for further processing. Tumour diagnosis was based on microscopic interpretation of a haematoxylin and eosin-stained 5 μm frozen tissue section. Fresh representative samples of all tissue components were fixed in 4% (v/v) formalin for paraffin embedding, or snap frozen in liquid nitrogen. Remaining tumour parts were dissociated in culture medium at room temperature, using two crossed scalp blades. Tissue fragments were allowed to settle in a 50 ml tube at 30 ml of culture medium. The supernatant, containing mostly single cells (as analysed by phase-contrast microscopy using a Zeiss Axiovert microscope), was washed twice with culture medium. To the cell suspension 10% (final volume) dimethyl sulphoxide was added slowly. The suspension was aliquoted, frozen in a Kryo 10 Series 2 automated freezer (Planer Biomed, Sunbury-on-Thames, UK) and stored in liquid nitrogen.

Tumour characterisation

Typing according to the WHO classification (Mostofi, 1980, 1984) was based on histology and immunohistochemical analysis of expression of germ cell-specific alkaline phosphatase (detected with antibodies to placenta-like alkaline phosphatase), α-fetoprotein, human chorionic gonadotropin (Dako, Glostrup, Denmark) and cytokeratins 8 and 18 (Beckton Dickinson, San Jose, CA, USA) using representative paraffin and frozen tissue sections (Oosterhuis et al., 1989).

Classification revealed 19 SEs and 19 NSs, the latter comprising five pure ECs, one MT, five YSs, one testicular Wilms' tumour of germ cell origin (Gillis et al., 1994) and seven mixed tumours. The mixed NSs comprised two tumours with EC, IT, MT and YS, one with IT, MT, YS and CC, one with MT and YS, and one with IT, MT and YS. Separate tumour nodules were used from two other mixed tumours; one with EC, IT, MT and YS besides EC with MT, the other with two SE nodules besides an EC component. The separate samples from these two tumours are referred to as T₁, T₂ and T₃ (for the latter), and are regarded as individual tumours. Besides the above-mentioned tumours, two normal parenchyma with active spermatogenesis and two abundantly CIS-containing parenchyma were analysed, as were a spermatocytic seminoma (SS), one YS derived from a xenografted mixed NS (TL37), one dermoid cyst and two testicular B-cell lymphomas.

Lymphocyte depletion

Cryopreserved single cell suspensions from five SEs, containing SE cells and lymphocytes, were rapidly thawed at 37°C, washed in 10 ml of culture medium and counted. The suspensions were treated with a 2.5-fold excess (relative to the total cell number) of magnetic beads coated with anti-CD2 monoclonal antibody (Dynal, Skoyen, Norway) to deplete lymphocytes. After 15–20 min incubation at room temperature with gentle shaking, 4 ml of culture medium was added, and the beads were removed using a magnetic particle collector (Dynal). The supernatant, containing enriched SE cells was removed. The beads were washed twice with culture medium and all supernatants were pooled. Removal of the lymphocytes was verified by microscopic examination of a cytospin preparation with haematoxylin and eosin staining. After treatment with magnetic beads, all suspensions contained less than 15% of lymphocytes.

Lyophilisation and glycolipid extraction

Similar packed cell volumes from untreated or bead-treated samples, as well as frozen tumour blocks of similar size were lyophilised overnight in a Freeze Mobile 12SL (Virtex Sentry, Gardner, USA). Upon lyophilisation, samples were sent to Philadelphia in numbered tubes, without any information on tumour histology to assure an objective assay. Glycolipids were extracted from an approximately equal packed volume of tumour cells using isopropyl alcohol–hexane–water (55:25:20, v/v/v), as described previously (Kannagi et al., 1982). Total lipid extracts were partitioned into an upper and lower phase according to the method of Folch-Pi et al. (1951). The upper phase was desalted using C18 reverse-phase columns (Analytichem, Harbor City, USA).

Glycolipid analysis

Major glycolipids (CMH, CDH, CTH) were detected using orcinol staining. Specific glycolipids were identified by co-

### Table 1 Glycolipid specificity of monoclonal antibodies used

| Antibody  | Glycolipid structure | Glycolipid name |
|-----------|----------------------|-----------------|
| Globo-series |                 |                 |
| b         | Galβ1→4Glcβ1→Cer   | CMH             |
| b         | Galα1→4Glcα1→Cer   | CTH             |
| 1A4-E10   | Galα1→4Galβ1→4Glcβ1→Cer | Glb3 (CTH) |
| MC630     | GalNAcβ1→3Galβ1→4Glcβ1→Cer | Gb4 (globoside) |
| MC630     | Galβ1→3GalNAcβ1→3Galβ1→4Glcβ1→Cer | Gb5 (SSA-3) |
| MC813     | NeuAca2→3Galβ1→4Glcα1→4Glcβ1→Cer | GL7 (SSA-3/4) |
| MC813     | GalNAcβ1→3Galβ1→3GalNAcβ1→3Galβ1→4Glcβ1→Cer | GL9 (SSA-3/4) |
| Lacto-series |                 |                 |
| MC480     | Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→Cer | Le* (SSEA-1) |
| MC480     | Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→Cer | extLe* (SSEA-1) |
| Ganglio-series |           |                 |
| R24       | NeuAca2→8NeuAca2→3Galβ1→4Glcβ1→Cer | GD3 |
| A2B5      | NeuAca2→8NeuAca2→3Galβ1→4Glcβ1→Cer | GT3 |

- Globo-series glycolipids contain Galα1→4Gal; lacto-series glycolipids contain GlcNAcβ1→3Gal; ganglio-series contain NeuAca2→3Gal. No antibodies to detect CMH and CDH are available; these molecules are detected using orcinol staining.
migration with pure glycolipid standards and by immuno staining with specific monoclonal anti-carbohydrate antibodies (Fenderson et al., 1987; Andrews et al., 1990). In brief, 5 μl of each glycolipid sample was streaked onto Whatman HP-FK silica gel plates and subjected to ascending chromatography using a solvent system of chloroform–methanol–water (50:40:10 v/v/v) containing 0.05% (w/v) calcium chloride. After drying, the chromatography plates were coated with 0.5% (w/v) polyisobutylmethacrylate (Aldrich, Milwaukee, MI, USA) in diethyl ether for 1 min, blocked for 2 h with 5% bovine serum albumin (BSA) (Sigma, St Louis, MO, USA) in phosphate-buffered saline (PBS), and then reacted with primary antibody overnight at 4°C. Bound antibody was detected using a 2 h incubation at 4°C with alkaline phosphatase-conjugated goat anti-mouse antibody (HyClone, Logan, USA) diluted 1:1000. Colour reaction was obtained through incubation with bromochloroindolyl phosphate (Fisher Biotech, NJ, USA) and nitroblue tetrazolium (Sigma) for 1 h at room temperature (Harlow and Lane, 1988).

Monoclonal antibodies

Anti-carbohydrate monoclonal antibodies (MAbs) were obtained and used as described previously (Fenderson et al., 1987). Gb3 was detected using MAb IA4-E10 (Fenderson et al., 1987); Gb4 and Gb5 were detected using MAb MC630 to SSEA-3 (Kanagi et al., 1983a); GL7 was detected using MAb MC813 to SSEA-4 (Kanagi et al., 1983b); Le* was detected using MAb MC480 to SSEA-1 (Solter and Knowles, 1978; Gooi et al., 1981); GD3 was detected using MAb R-24 (Dippold et al., 1984); GT3 was detected using MAb A2B5 (Eisenbarth et al., 1979). The glycolipid carbohydrate structures recognised by these reagents are listed in Table I.

Gangliosides are designated according to the nomenclature of Svennerholm (1964). Glycolipids are designated according to the recommendations of the IUPAC Nomenclature Committee (IUPAC-IUB, 1978).

Results

Glycolipid profiles of lymphocyte-depleted seminoma cell suspensions

SEs are known to contain infiltrating lymphocytes (Mostofi, 1980, 1984), which could influence our tumour glycolipid analysis. Therefore, magnetic anti-CD2 coated beads were used to remove these inflammatory cells from SE cell suspensions. Thin-layer chromatography and subsequent orcinol or immunostaining for SSEA-1, SSEA-3 and SSEA-4, using pellets of either untreated or lymphocyte-depleted cells, revealed that lymphocyte depletion did not result in a marked change in glycolipid profile (Figure 1). Orcinol staining revealed an additional band of unknown origin in the bead-treated samples that did not react with any of the MAbs included in this study. Whether this band is specific for SEs needs further investigation. Gb3 and Gb4 were the major glycolipids in all five SE samples. Two tumours, TL1049 and TL3544, were found to have high levels of glycolipid expression. These tumours contained an extended GL7 glycolipid, referred to as GL9, previously shown to be present in NT2 cells (Andrews et al., 1990).

Glycolipid profiles of intact tumour tissues

Lymphocytes in SEs did not interfere with our glycolipid analysis. Because of this finding and as expression of certain glycolipids has been found immunohistochemically to occur...
specifically in CIS and TGCT cells (Kang et al., 1995), we assumed that non-malignant cells in NSs would not interfere with the glycolipid analysis either. Therefore, we proceeded to use lyophilised tissue samples from snap frozen tumours for subsequent analyses. The results of our orcinol and immunostaining analyses are shown in Figure 2. All data concerning the glycolipid profiles of the 50 analysed samples are listed in Table II and summarised in Table III.

Compared with normal testicular parenchyma, CIS-containing parenchyma was characterised by the abundant presence of Gb3 and Gb5, and an increase in the expression of Gb4 and GL7.

Of 21 SEs analysed, all tumours expressed the globo-series glycolipid GL7, whereas CDH was found in nine, Gb3 and Gb4 in 19 and Gb5 in ten SEs. The ganglio-series glycolipids GD3 and GT3 were present in 14 and ten SEs respectively. The expression level of the distinct glycolipids varied among the SEs. With regard to GL7 in particular, two groups of SEs could be distinguished: one with a low and one with a high level of expression. Since tumour cell enrichment by lymphocyte depletion did not result in a marked change in detection levels of the glycolipids and similar-size tumour blocks were used for glycolipid extraction, the high and low glycolipid levels found in the tumour blocks apparently reflect differences in expression level and not a variation in the amount of tumour cells present in each sample.

In contrast to the SEs, only one of which expressed Lea, all ECs contained this marker. CDH and Gb5 were also present in all ECs. These tumours further expressed Gb3, Gb4 and GL7 in all samples, as did the majority of the SEs. Two ECs were found to weakly express GD3, whereas only one tumour contained GT3.

The YSs did not display a clearly defined glycolipid profile. One tumour expressed Gb3, Gb4, Gb5, GL7 and Le1. Two tumours expressed Gb3, Gb4 and GD3, either in combination with Le1 or GT3. One tumour expressed Gb5, GL7, Le1 and GD3. Two YSs completely lacked all four globo-series glycolipids: one contained Le1 only, while the other, derived from a xenografted mixed tumour, had GD3 and GT3.

Compared with SEs and ECs, the nine (mixed) NSs had reduced levels of globo-series glycolipids, especially Gb3 and Gb5, whereas CDH and Le1 were found in the majority of the samples. Eight NSs contained GD3 and GT3. The highest gangloside levels were found in tumours with at least an MT component. The pure MT had trace amounts of Gb3, Gb4 and GL7, besides high levels of GD3 and GT3.

The SS did not express GL7 and Le1. The dermoid cyst contained Gb3, Gb4, GL7, GD3 and GT3. One B-cell lymphoma contained some CDH, whereas the other had low levels of CDH, Gb4, GL7 and Le1.

Discussion

We analysed whether the glycolipid content of human NS cell lines reflects the situation in primary tumours, using thin-layer chromatography and carbohydrate immunostaining. We
also analysed the glycolipid content of CIS and SEs, particularly to reveal the relationship of the latter with ECs. SEs and testicular parenchyma containing CIS were characterised by similar glycolipid patterns. This result attests to the phenotypic similarity of CIS and SE cells.

As expression of ganglosides is regarded as a marker of differentiation (Fenderson et al., 1987), the finding of GD3 and GT3 in many SEs confirms the thought that SEs form a heterogeneous population. It can be speculated that the ganglioside-containing SE cells are derived from precursor

| No. | Tumour | CDH | Gb3 | Gb4 | Gb5 | GL7 | Le<sup>e</sup> | GD3 | GT3 |
|-----|--------|-----|-----|-----|-----|-----|------------|-----|-----|
| CIS and seminomas | | | | | | | | | |
| 13. | TL1804 (CIS/SE) | + | + | + | + | + | + | + |  
| 15. | TL3724 (CIS/NS) | + | + | + | + | + | + | + |  
| 1. | TL7573 | + | + | + | + | + | + | + |  
| 2. | TL614 | + | + | + | + | + | + | + |  
| 14. | TL3174 | + | + | + | + | + | + | + |  
| 15. | TL287 | + | + | + | + | + | + | + |  
| 16. | TL8225 | + | + | + | + | + | + | + |  
| 19. | TL2207T3 | + | + | + | + | + | + | + |  
| 26. | TL1487 | + | + | + | + | + | + | + |  
| 27. | TL229 | + | + | + | + | + | + | + |  
| 29. | TL2207T1 | + | + | + | + | + | + | + |  
| 37. | TL8837 | + | + | + | + | + | + | + |  
| 38. | TL9089 | + | + | + | + | + | + | + |  
| 40. | TL189 | + | + | + | + | + | + | + |  
| 41. | TL8763 | + | + | + | + | + | + | + |  
| 42. | TL74 | + | + | + | + | + | + | + |  
| 45. | TL8888 | + | + | + | + | + | + | + |  
| 47. | TL539 | + | + | + | + | + | + | + |  
| 48. | TL1049 | + | + | + | + | + | + | + |  
| 49. | TL3544 | + | + | + | + | + | + | + |  
| 50. | TL8285 | + | + | + | + | + | + | + |  
| 51. | TL9244 | + | + | + | + | + | + | + |  
| 52. | TL4873 | + | + | + | + | + | + | + |  
| Embryonal carcinomas | | | | | | | | | |
| 5. | TL5207 | + | + | + | + | + | + | + |  
| 7. | TL2207T2 | + | + | + | + | + | + | + |  
| 17. | TL3635 | + | + | + | + | + | + | + |  
| 28. | TL524 | + | + | + | + | + | + | + |  
| 43. | TL269 | + | + | + | + | + | + | + |  
| 46. | TL87 | + | + | + | + | + | + | + |  
| Yolk sac tumours | | | | | | | | | |
| 30. | TL37R21 | + | + | + | + | + | + | + |  
| 40. | TL1013 | + | + | + | + | + | + | + |  
| 4. | TL7873 (MT)<sup>a</sup> | + | + | + | + | + | + | + |  
| 9. | TL6322 (EC)<sup>a</sup> | + | + | + | + | + | + | + |  
| 25. | TL1973 (EC,IT)<sup>a</sup> | + | + | + | + | + | + | + |  
| 36. | TL7162 (EC)<sup>a</sup> | + | + | + | + | + | + | + |  
| Non-seminomas | | | | | | | | | |
| 8. | TL6745 (MT) | + | + | + | + | + | + | + |  
| 10. | TL3819 (IT,MT,YS,CC) | + | + | + | + | + | + | + |  
| 11. | TL3035 (MT,YS) | + | + | + | + | + | + | + |  
| 22. | TL3771 (EC,IT,MT,YS) | + | + | + | + | + | + | + |  
| 23. | TL6936 (MT,IT,YS) | + | + | + | + | + | + | + |  
| 31. | TL189 (WT) | + | + | + | + | + | + | + |  
| 32. | TL1348 (EC,IT,MT,YS) | + | + | + | + | + | + | + |  
| 33. | TL37T2 (MT,EC) | + | + | + | + | + | + | + |  
| 34. | TL8007 (EC,IT,MT,YS) | + | + | + | + | + | + | + |  
| Spermatocytic seminoma | | | | | | | | | |
| 44. | TL8743 | + | + | + | + | + | + | + |  
| Non-germ cell tumours | | | | | | | | | |
| 3. | TL8558 (DC) | + | + | + | + | + | + | + |  
| 18. | TL4224 (L) | + | + | + | + | + | + | + |  
| 20. | TL6661 (L) | + | + | + | + | + | + | + |  
| Testicular parenchyma | | | | | | | | | |
| 12. | TL1540 | + | + | + | + | + | + | + |  
| 24. | TL1541 | + | + | + | + | + | + | + |  

Results represent a synthesis of thin-layer chromatography orcinol and immunostaining data. The scale is negative (no symbol) to strong positive (+ + + +). Le<sup>e</sup> antigen was carried on multiple glycolipid species. CC, choriocarcinoma; CIS/SE, CIS/NS, carcinoma in situ-containing testicular parenchyma adjacent to a seminoma or non-seminoma respectively; DC, dermoid cyst; EC, embryonal carcinoma; IT, immature teratoma; L, lymphoma of the testis; MT, mature teratoma; YS, yolk sac tumour. *Four YSs contained minor amounts of non-YS cells, as indicated; WT, testicular Wilms' tumour of germ cell origin.
Table III Glycolipid expression in human germ cell tumours

| Glycolipid | N (2) | CIS/SE (23) | Cell type | EC (6) | YS (60) | NS (8) |
|-----------|------|------------|-----------|--------|---------|--------|
| Globo-series |      |            |           |        |         |        |
| CDH       | 1, + | 11, +      | 6, + +    | 3, +   | 6, + +  |        |
| Gb3       |      |            |           |        |         |        |
| Gb4       | 2, + | 22, + +    | 6, + ++   | 3, +   | 7, + +  |        |
| Gb5       |      |            |           |        |         |        |
| GL7       | 2, + | 23, + + +  | 6, + +    | 2, +   | 5, +   | 7, +   |
| Lacto-series |     |            |           |        |         |        |
| Le        | 1, + | 6, + +     | 4, + +    | 6, + + |         |        |
| Ganglio-series |    |            |           |        |         |        |
| GD3       | 1, + | 15, + + +  | 2, +      | 4, + + | 7, + +  |        |
| GT3       |      | 11, +      |           | 2, +   | 7, +   |        |

The number of samples (of the total number analysed, indicated in brackets) expressing the indicated marker and the average immunostaining intensity are shown. Glycolipid structures were identified in this report by: (i) co-migration on thin-layer chromatography plates with pure glycolipid standards and (ii) by immunostaining using specific anti-glycolipid monoclonal antibodies. CIS, carcinoma in situ-containing testicular parenchyma; EC, embryonal carcinoma; N, normal testicular parenchyma; NS, non-seminomatous testicular germ cell tumour; SE, seminoma; YS, yolk sac tumour. *Four YSs contained minor amounts of other non-seminomatous cell types, as indicated in Table II. The results of a testicular Wilm’s tumour were not included in the average staining intensity of non-seminomas. Expression is from absent (no symbol) to strong (+ + +).
cell line NT2 (Wenk et al., 1994). EC cells express almost exclusively large amounts of globo-series glycolipids (apart from Leb). The NSs with differentiated components are characterised by a lower expression of the globo-series glycolipids, especiallyGb3 andGb5, expression of the lacto-series glycolipid Lea in the majority of the tumours and presence of the gangliosides GD3 and GT3, at the highest levels in tumours with at least an MT component. These data confirm the morphological observations of the presence of a minor stem cell population in differentiated NSs. Although our semi-quantitative analysis of the glycolipid expression in pure tumours indicates which glycolipids are expressed by the various cell types, an immunohistochemical approach could be used to study the distribution of glycolipids, especially concerning the non-seminomatous cell types in mixed tumours.

Our data on the spermatocytic seminoma support the contention that this tumour type is a separate GCT entity, not derived from CIS cells (Burke and Mostofi, 1993; Cummings et al., 1994). Based on their glycolipid content, the spermatocytic seminoma, non-GCTs and the normal parenchyma of the testis could readily be discriminated from TGCTs and parenchyma containing CIS.

In conclusion, our analysis of the glycolipid content of human primary TGCTs confirms the data obtained on non-seminomatous cell lines (Wenk et al., 1994). Globo-series glycolipids are highly expressed in ECs, whereas the expression of especiallyGb3 andGb5 is reduced in differentiated non-seminomatous elements. In addition, we show that the globo-series glycolipids are expressed at similar levels in CIS, SEs and ECs. The expression of Leb by ECs allows discrimination between this tumour type and SEs, which do not express this marker. Gangliosides are found in many SEs and almost all differentiated NSs, but are rare in ECs. These results could be integrated in the speculative model shown in Figure 3. Primitive cells, i.e. CIS and SE cell, are characterised by globo-series glycolipids. These tumour cells could develop along two pathways. Either they mature (differentiate) in the germ cell lineage and start expressing gangliosides, or they are reprogrammed to become pluripotent EC cells and start expressing lacto-series Lea. When these reprogrammed cells mature (differentiate) into various lineages they start expressing gangliosides as well. The present data fit into the linear progression model, but do not prove it. Studies comparing the glycolipid profile of CIS and adjacent tumour, either SE or NS, should be performed to further investigate this model. In addition, it would be interesting to see if modulation of the glycolipids can change the phenotype of the tumour cells. Preliminary studies with NT2 (EC) cells using the glycosyltransferase inhibitor PDMP indicate that glycolipid depletion results in changed growth and shape of the cells (unpublished observations). This issue might also be addressed using transfection with glycosyltransferase genes to change glycolipid patterns. At present, these studies have to be limited to non-seminomatous cell types, as SE cell lines are not available.

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References

ANDREWS PW, BRONSON DL, BENHAM F, STRICKLAND S AND NOWLES BB. (1980). A comparative study of eight cell lines derived from human testicular teratocarcinoma. Int. J. Cancer, 26, 269—280.

ANDREWS PW, NUDELMAN E, HAKOMORI S AND FENDERSON BA. (1990). Different patterns of glycolipid antigens are expressed following differentiation of TER-2 human embryonal carcinoma cells induced by retinoic acid, hexamethylene bisacetamide (HMBA) or bromodeoxyuridine (BUdR). Differentiation, 43, 131—138.

ANDREWS PW, CASPER, J, DAMJANOV, J, DUGGAN-KEEN M, GOWERCMA A, HATA J, VON KEITZ A, LOOJENGA LHI, OOSTERHUIS JW, PERA M, SAWADA M, SCHMOLL H-J, SKAKKERBAEKEN NE, VON PUTTEN W AND STERN P. (1996). A comparative analysis of cell surface antigens expressed by cell lines derived from human germ cell tumours. Int. J. Cancer. (in press).

BIRD J AND KIMBER SJ. (1984). Oligosaccharides containing fucose linked σ(1—3) and σ(1—4) to N-acetylgalactosamine cause decapsulation of mouse morulae. Dev. Biol., 104, 449—460.

BREMER EG, HAKOMORI S, BOWEN-POPE DF, RAINES E AND ROSS R. (1984). Ganglioside-mediated modulation of cell growth, growth factor binding and receptor phosphorylation. J. Biol. Chem., 259, 6818—6825.

Glycolipids of germ cell tumours

Glycolipids of germ cell tumours

BURKE AP AND MOSTOFI FK. (1993). Spermatocytic seminoma. A clinicopathologic study of 79 cases. J. Urol Pathol., 1, 21—32.

CASPER J, SCHMOLL H-J, SCHNADT U AND FONATSCH C. (1987). Cell lines of human germinal cancer. Int. J. Androl., 10, 105—113.

CUELLO AC, GAROFALO L, KENIGSBRG RL AND MAYSINGER D. (1989). Gangliosides potentiate in vivo and in vitro effects of nerve growth factor on central cholinergic neurons. Proc. Natl Acad. Sci. USA, 86, 2056—2060.

CUMMINGS OW, ULBRIGHT TM, EBLE JN AND ROTH LM. (1994). Spermatocytic seminoma: an immunohistochemical study. Hum. Pathol., 25, 54—59.

CZAJA IT AND ULBRIGHT TM. (1992). Evidence for the transformation of seminoma to yolk sac tumor, with histogenetic considerations. Am. J. Clin. Pathol., 97, 468—477.

CZERNOBILSKY B. (1991). Differentiation patterns in human testicular germ cell tumours. Virchows Arch. A Path. Anat. Histol., 419, 77—78.

DAMJANOV I, FOX N, NOWLES BB, SOLTER, D, LANGE PH AND FRALEY EE. (1982). Immunohistochemical localization of murine stage-specific embryonic antigens in human testicular germ cell tumors. Am. J. Pathol., 108, 225—230.
Glycolipids of germ cell tumours

RA Olie et al

mostofi fk. (1980). pathology of germ cell tumors of testis. a progress report. cancer, 45, 1735–1754.
mostofi fk. (1984). tumour markers and pathology of testicular tumours. in progress and controversies in oncological urology, 49–87. ar, liss; new york.
mostofi fk, sesterhenia ia and davis cj. (1987). immunopathology of germ cell tumors of the testis. semin. diag. pathol., 4, 320–41.
motzer rj, reuter ve, cordon-carbo c and bosl gj. (1988). blood group-related antigens in human germ cell tumors. cancer res., 48, 5342–5347.
ohyama c, fukushi y, satoh m, saito sh, orikasa s, nuedelmann e, straub m and hakomori s. (1990). changes in glycolipid expression in human testicular tumor. int. j. cancer, 45, 1040–1044.
ohyama c, orikasa s, satoh m, saito s, ohtani h and fukushi y. (1992). globotriaosylceramide glycolipid in seminoma: its clinicopathological importance in differentiation from testicular malignant lymphoma. j. urol., 148, 72–75.
oile ra, looijenga ljh, boerjigter l, top b, rodenhuis s, mulder mp and oosterhuis jw. (1995a). n- and kras mutations in human testicular germ cell tumors: incidence and possible biological implications. genes chrom. cancer, 12, 110–116.
oile ra, looijenga ljh, dekker mc, de jong fh, de rooy dg and oosterhuis jw. (1995b). heterogeneity in the in vitro survival and proliferation of human seminoma cells. br. j. cancer, 71, 1590–1591.
oplier rtd. (1987). hla phenotype and clinicopathological behaviour of germ cell tumours: possible evidence for clonal evolution from seminomas to non-seminomas. int. j. androl., 10, 95–93.
oplier rtd. (1990). clues from natural history and results of treatment supporting the monoclonal origin of germ cell tumours. cancer surv., 9, 333–368.
oosterhuis jw, de jong b, van dalen i, van der meer i, visser m, de leu l, mesander g, collard jg, scharff ford kops h and slevier dm. (1985). identical chromosomal translocations involving the region of the c-myc oncogene in four metastases of a mediastinal teratocarcinoma. cancer genet. cytogenet., 15, 99–107.
oosterhuis jw and looijenga lh. (1993). the biology of human germ cell tumours: retrospective speculations and new perspectives. eur. urol., 23, 245–250.
oosterhuis jw, castedo smj, de jong b, cornelisse cj, dam a, slevier dt and scharff fordt kops h. (1989). ploidy of primary germ cell tumours of the testis. pathogenetic and clinical relevance. lab. invest., 60, 14–20.
pattillo ra, ruckert a, hussa r, bernstein r and defles e. (1971). the jar cell line. continuous human multihormone production and controls. in vitro, 6, 398–399.
perera mf, blasco lafita mj and mills j.i. (1978). cultured stem cells from human testicular teratomas: the nature of human embryonal carcinoma, and its comparison with two types of yolk sac carcinoma. int. j. cancer, 40, 334–343.
pierce gb and abell mr. (1970). embryonal carcinoma of the testis. pathol. annu., 5, 27.
sesterhenia ia. (1985). the role of intratubular malignant germ cells in the histogenesis of germ cell tumors. in proceedings of the 2nd germ cell tumor conference leeds 8–10 september 1993, jones wg, milford ward a and anderson ck. (eds), pp. 25–35, leeds.
skakkebekk ne, berthelson gj, giwercman a and müller 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.
solter d and knowles bb. (1978). monochlonal antibody defining a stage-specific mouse embryonic antigen (ssea-1). proc. natl. acad. sci. usa, 75, 5565–5569.
svennerholm l. (1964). the gangliosides. j. lipid res., 5, 145–162.
von keitz a, riedmiller h, neumann k, gutschank w and fonatsch c. (1995). establishment and characterization of human seminoma clines. invest. urol. (in press).
wenk j, andrews wp, casper j, mata h, pera mf, von keitz a, damjanov a and fenderon ba. (1994). glycolipids of germ cell tumors: extended globo-series glycolipids are a hallmark of human embryonal carcinoma cells. int. j. cancer, 56, 108–115.