Glycosylation & Disease, 1994, **1**, 119–125

Type 2 chain glycosylation in thyroid carcinomas

Magne Bryne*†, Erik Dabelsteen‡ and Jahn M. Nesland*

**The Norwegian Radium Hospital, Institute of Cancer Research, Oslo, Norway; †Norwegian Cancer Society, Oslo, Norway; ‡Department of Oral Diagnostics, University of Copenhagen, Denmark*

Alterations of cell-surface carbohydrates are important for the metastatic behaviour of human carcinomas. Follicular and papillary thyroid carcinomas clinically show a very different metastatic pattern and represent a good model for studies of the metastatic process. We examined the expression of various Le^x-related carbohydrates in 30 primary papillary, 20 primary follicular and 15 primary anaplastic thyroid carcinomas by means of immunohistochemistry. Six metastases from papillary and five from follicular carcinomas were also examined. Morphologically normal thyroid epithelium did not express any of the type 2 carbohydrates. Papillary carcinomas were immunoreactive for several type 2 carbohydrates, including sialyl-Lewis X, in contrast to follicular and anaplastic carcinomas. The metastases showed no significant differences in expression of carbohydrates compared with the primary tumours. We hypothesize that the up-regulation of sialyl-Lewis X and some other related carbohydrates in papillary carcinomas is of importance for the clinical behaviour of these tumours.

Keywords: carbohydrates, Lewis antigens, thyroid carcinomas

Introduction

Several studies have shown that changes in cell-surface carbohydrates (CHs) are important for the invasive and metastatic behaviour of human carcinomas [1–3]. The function of these CHs is virtually unknown, but there is accumulating evidence that they are cell–cell and cell–matrix recognition molecules [1, 3–7]. Thus, several studies have focused upon oligosaccharides as diagnostic markers and as potential targets in cancer treatment [2, 3, 8–10].

Papillary and follicular thyroid carcinomas have different metastatic properties. Papillary thyroid carcinomas mainly metastasize to lymph nodes, whereas follicular thyroid carcinomas mainly spread haematogenously [11, 12]. The biological explanation for this different organ-specific metastatic behaviour is unknown [12, 13].

In this study we have examined the distribution of several type 2 chain CHs, some of which have recently been suggested to be adhesion molecules (for example sialyl-Le^x) and of significance for the metastatic process [1, 5, 14, 15]. We show that papillary and follicular thyroid carcinomas exhibit differential expression of various Le^x-related type 2 CHs.

Address correspondence to: M. Bryne, Institute of Cancer Research, The Norwegian Radiumhospital, Montebello, 0310 Oslo, Norway. Tel: (+47) 22934630; Fax: (+47) 22730164.

Materials and methods

Tissue

The material consisted of 65 thyroid carcinomas (30 papillary, 20 follicular and 15 anaplastic). Lymph node metastases from six of the primary papillary tumours and five distant metastases from follicular carcinomas (two lung and three bone) were included. The tissues were fixed in 10% buffered formalin and paraffin embedded. All cases were obtained from the files of Department of Pathology, The Norwegian Radium Hospital, Oslo, Norway.

Antibodies

Six monoclonal anti-carbohydrate antibodies reacting with type 2 CHs with different specificities were used. The antigens studied were as follows:

Table 1. Primary monoclonal antibodies used, their isotype, dilution and chemical structure of the carbohydrate antigens

Antibody [source ref.]	Isotype	Antigen	Chemical structure of antigen	Dilution[a]
IB2 [16]	IgM	LAC	Galβ1–4GlcNAcβ1-R	1:10
BE2 [16]	IgM	H	(Fucα1–2)Galβ1–4GlcNAcβ1-R	1:10
SH1 [17]	IgG	Le^x	Galβ1–4(Fucα1–3)GlcNAcβ1-R	1:7
AH6 [18]	IgM	Le^y	(Fucα1–2)Galβ1–4(Fucα1–3)GlcNAcβ1-R	1:10
SNH3[b]	IgM	s-Le^x	(NeuAcα2–3)Galβ1–4(Fucα1–3)GlcNAcβ1–4 (Fucα1–3)GlcNacβ1-R	1:10

[a]Diluted from a hybridoma supernatant with an immunoglobulin content of 10–30 μg/ml.
[b]A. Singhal *et al.*, unpublished results.

N-acetyllactosamine (LAC), sialyl-*N*-acetyllactosamine (s-LAC), blood group H structure (H), Lewis X (Le^x), Lewis Y (Le^y) and sialyl-Lewis X (s-Le^x). The specificities of the antibodies, isotypes, dilutions and sources are shown in Table 1.

Immunohistochemical staining
Four-micron sections were placed on gelatin–chrome–alum-coated slides, deparaffinized in xylene and brought to water through a 100% and a 96% solution of ethyl alcohol. An avidin–biotin–peroxidase complex (ABC) method was applied, using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) as previously described [19].

To remove terminal sialic acid from the LAC carbohydrate antigen, sections were incubated for 2 h at 37°C with neuraminidase (Test-Neuraminidase, ORKD 04/05, 20 μl in 1 ml 0.1 M sodium acetate buffer, pH 5.5; Behringwerke, Marburg, Germany) before application of the primary antibody, 1B2.

Control staining reactions included replacement of the primary antibody with (i) phosphate-buffered saline (PBS), (ii) monoclonal antibody of another and irrelevant specificity, but of the same isotype, and (iii) a culture supernatant of the myeloma cell line (SP2) used for hybridization. Endothelial cell staining was an inherent positive control for expression of H and s-LAC. In addition, sections from various carcinomas known to express these CHs were used as positive controls.

Scoring of immunostaining
The fraction of immunostained cells in the whole tumour was registered semiquantitatively. Staining was recorded as: 0 = no cells positive, + = less than 5% cells positive, ++ = 5–30% cells positive, +++ = more than 30% cells positive. Staining was recorded as located either in the cytoplasm or on the cell-surface membrane. The intensity of immunostaining was not recorded.

Results

The expression of type 2 carbohydrates is shown in Table 2 and Figures 1–5. s-Le^x, Le^x, LAC, s-LAC and Le^y were expressed in a variable number of papillary tumours. The Le^y structure was the only carbohydrate expressed in follicular carcinomas except that one follicular carcinoma also showed a few LAC-positive cells. In anaplastic carcinomas half of the tumours expressed Le^y and 20% showed a few LAC-positive cells. The staining pattern in metastases was the same as in the primary tumours. No obvious relation between reactivity for different CHs in papillary thyroid carcinomas was seen. The staining of tumour cells in papillary tumours was generally located on the cell surface (Figures 1–4). In most of the follicular thyroid carcinomas and anaplastic carcinomas that expressed CHs a cytoplasmic immunoreactivity was seen (Figure 5).

In four primary tumours (two papillary and two follicular) we detected tumour cells in blood vessels. In papillary thyroid carcinomas, these intravascular tumour cells were immunoreactive for s-Le^x, Le^y, Le^x, LAC and s-LAC on the surface (Figure 4). The intravascular cells in the two follicular carcinomas did not express any of the CH structures examined.

Adjacent normal-looking thyroid epithelium did not express any of the CH structures (Figure 1).

Table 2. Immunostaining of some type 2 carbohydrate structures in normal thyroid and different thyroid carcinomas

Tissue	Extent of staining[a]	Carbohydrate structures (per cent of tumours)					
		LAC	s-LAC	H	Le^x	s-Le^x	Le^y
Normal thyroid			–	–	–	–	–
Primary papillary tumours[b]	+	27	63	–	33	70	30
(n = 30)	++	–	7	–	–	–	37
	+++	–	–	–	–	–	23
Papillary tumours[b]	+	67	33	–	67	67	17
(metastases)	++	–	33	–	–	–	33
(n = 6)	+++	–	–	–	–	–	33
Primary follicular tumours[c]	+	5	–	–	–	–	15
(n = 20)	++	–	–	–	–	–	–
	+++	–	–	–	–	–	10
Follicular tumours[c]	+	–	–	–	–	–	25
(metastases)	++	–	–	–	–	–	–
(n = 5)	+++	–	–	–	–	–	–
Anaplastic carcinomas[c]	+	–	20	–	–	–	33
(n = 15)	++	–	–	–	–	–	13
	+++	–	–	–	–	–	7

[a]–, no cells positive; +, < 5% cells positive; ++, 5–30% cells positive; +++, > 30% cells positive.
[b]Generally membrane staining.
[c]Generally cytoplasmic staining.

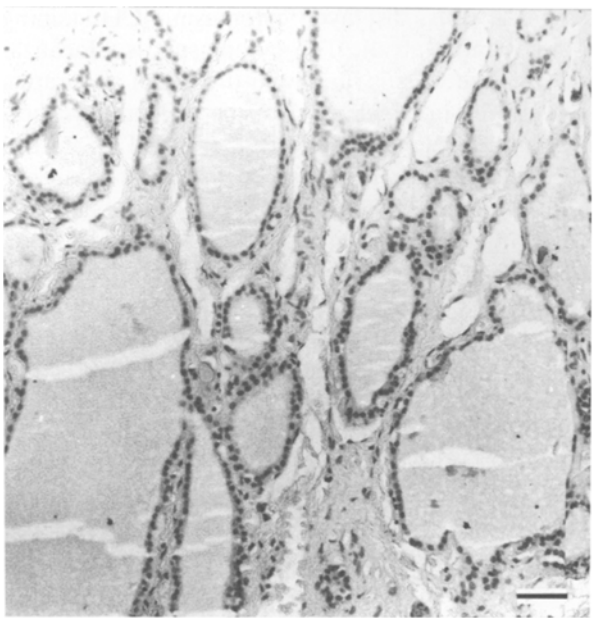

Figure 1. Morphologically normal thyroid tissue showing no expression of any of the studied type 2 carbohydrates. Bar = 100 μm.

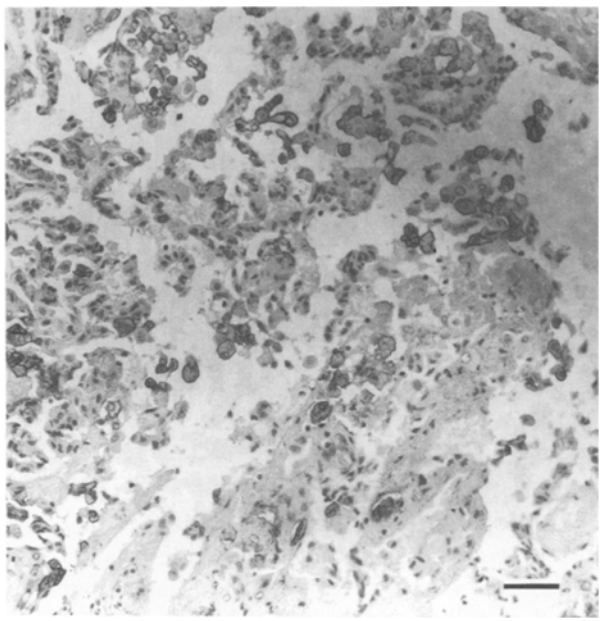

Figure 2. Primary papillary thyroid carcinoma showing a subpopulation of cells expressing Le^y oligosaccharide on the cell surface. Bar = 50 μm.

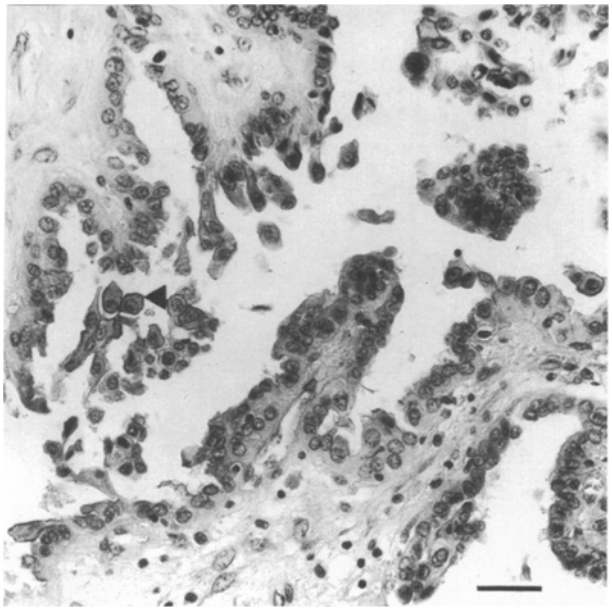

Figure 3. Primary papillary thyroid carcinoma showing a subpopulation of cells expressing s-LAC oligosaccharide on the cell surface (arrowhead). Follicular carcinomas did not express this structure. Bar = 30 μm.

Discussion

It is well documented that papillary tumours spread almost exclusively to lymph nodes and that follicular tumours generally spread haematogenously [12]. In this work we found that cells in papillary tumours expressed several Le^x-related CH structures, in striking contrast to follicular tumours.

The molecular mechanisms involved in the arrest of cancer cells in lymph nodes are virtually unknown [20]. A few recent *in vitro* adhesion studies, however, suggest that various integrins and some oligosaccharides are important in the adhesion of cancer cells to lymphatic extracellular matrix and possibly to lymphatic endothelium [20, 21]. It has been suggested that some of the CH structures detected in papillary tumours function as cell adhesion molecules *in vivo* [17, 22–25]. There is, however, no experimental evidence at present that these Le^x-related CHs are adhesion molecules in the lymphatic metastatic process. The present work suggests, on the other hand, that these CHs are not involved in the haematogenous spread of follicular tumours. This is in contrast to what is probably the case for colorectal carcinomas [26].

Expression of a few, but not all, of the blood group-related CHs studied in the present work has previously been investigated in thyroid tissue [27–29]. Our results confirm that normal thyroid tissue does not show immunoreactivity for these blood group-related type 2 chain CHs. We and others [27–29] have found striking glycosylation differences between normal and malignant thyroid tissue, confirming that an altered CH pattern is correlated with malignant transformation in epithelial tissues [1, 3, 30].

Several studies have shown a prognostic value for various CHs in different human carcinomas [2, 19, 26, 31]. The Le^y oligosaccharide is strongly expressed in the majority of carcinomas (especially papillary tumours) [27]. However, we observed considerable individual differences in the immunostaining for this structure. Further studies are needed to elucidate the role of Le^y and other CHs as prognostic markers for thyroid neoplasms.

Most anaplastic thyroid carcinomas are derived from the same epithelial cells as papillary and follicular tumours [12], and often minor tumour areas with either papillary or follicular morphology are seen. In this study we found that papillary cells in particular expressed CHs in a very different pattern from the adjacent anaplastic cells, supporting previous studies that expression of CHs is highly associated with cellular differentiation [1, 3, 32]. A general finding was that CHs in papillary cells were detected on the cell surface, whereas the follicular cells, and especially the anaplastic cells, displayed cytoplasmic CH staining or no staining at all. The cytoplasmic sublocalization of these CHs is mainly in the Golgi apparatus, an organelle in which most of the CHs are synthesized, and in secretory vesicles [33, 34]. This shift in subcellular distribution of CH in highly differentiated to poorly differentiated cells has been observed in various carcinomas [16, 34, 35]. The biological significance of the retention of CHs in cytoplasm is unknown [34]. It is, however, unlikely that CHs in cytoplasm are involved in the adhesive properties of the cells.

Lack of immunoreactivity for a CH structure in cancer tissue indicates either (a) little or no biosynthesis or (b) an elongated or masked molecule [3, 30]. Thus, our observations that type 2 carbohydrates were not expressed by normal thyroid tissue or on the surface of the cells in follicular and anaplastic carcinomas does not necessarily mean that the biosynthesis of CH is similar. We believe that it is much more likely that significant differences in various glycoproteins or glycolipids on the cell surface will be found between normal

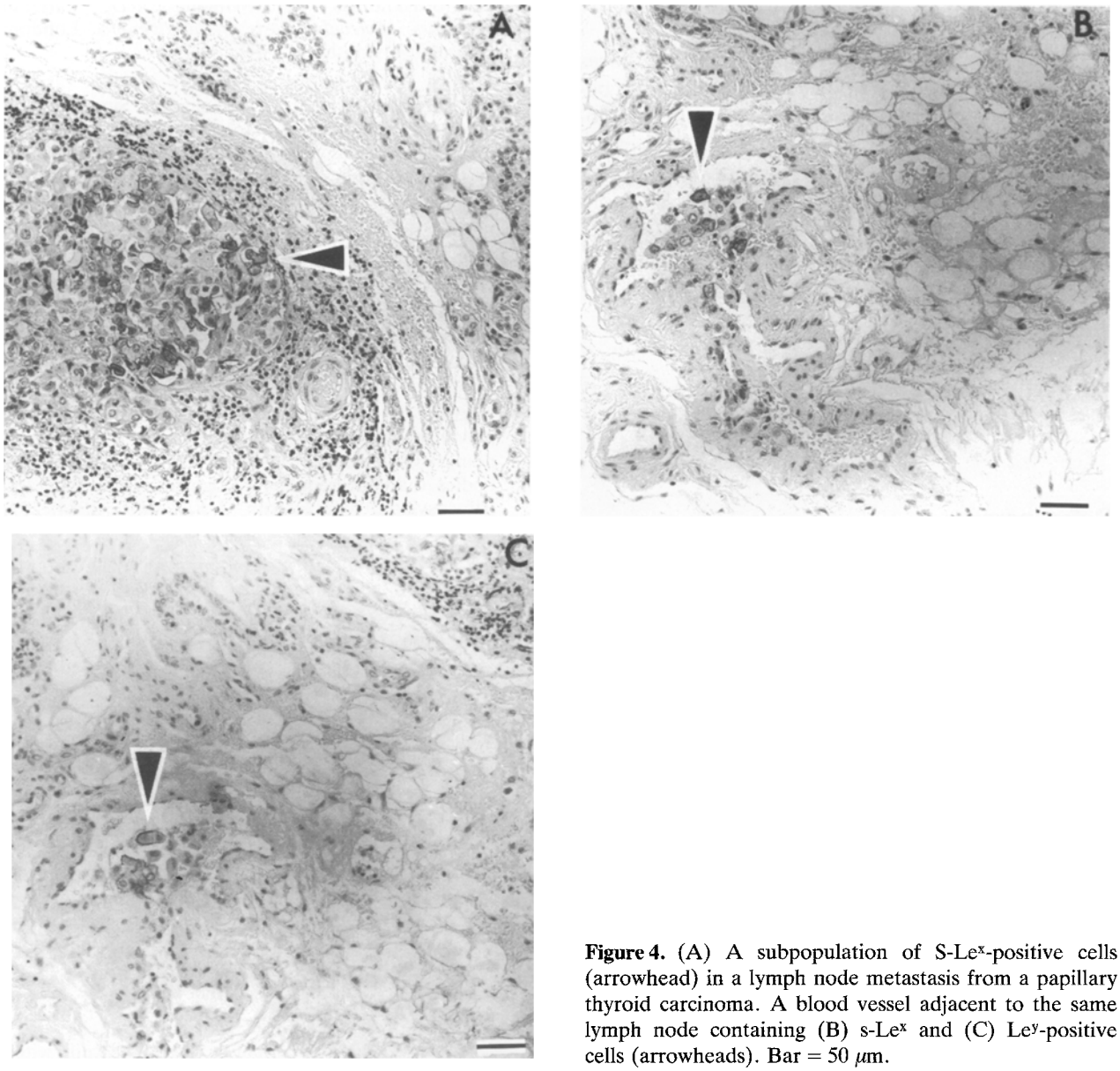

Figure 4. (A) A subpopulation of S-Le^x-positive cells (arrowhead) in a lymph node metastasis from a papillary thyroid carcinoma. A blood vessel adjacent to the same lymph node containing (B) s-Le^x and (C) Le^y-positive cells (arrowheads). Bar = 50 μm.

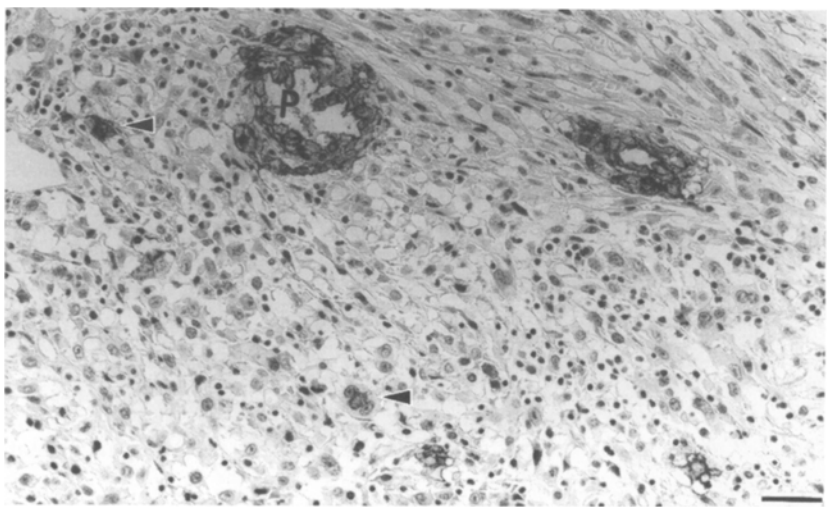

Figure 5. Anaplastic thyroid carcinoma probably derived from a papillary carcinoma. Le^y oligosaccharide is expressed on cell membranes on moderately to highly differentiated cells (P) in contrast to no staining or granular cytoplasmic staining (arrowheads) in anaplastic cells. Bar = 50 μm.

thyroid tissue and these carcinomas using more sensitive methods [3].

In conclusion, we found striking CH differences between papillary and follicular tumours and between normal thyroid tissue and thyroid carcinomas. We hypothesize that these differences in cell-surface CHs are partly responsible for the different pattern of metastatic behaviour in papillary and follicular thyroid carcinomas.

Acknowledgements

We thank Ellen Hellesylt, Mette Myhre and Liv Inger Håseth for skillful assistance.

References

1. Varki A. Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology* 1993: **3**; 97–130.
2. Muramatsu T. Carbohydrate signals in metastasis and prognosis of human carcinomas. *Glycobiology* 1993: **3**; 294–6.
3. Hakomori S. Abberant glycosylation in tumors and tumor-associated carbohydrate antigens. *Adv Cancer Res* 1989: **52**; 257–331.
4. Varki A, Hooshmand F, Diaz S, Varki NM, Hedrick SM. Development abnormalities in transgenic mice expressing a sialic acid-specific 9-*O*-acetylesterase. *Cell* 1991: **65**; 65–74.
5. Hakomori S. Possible functions of tumor-associated carbohydrate antigens. *Curr Opin Immunol* 1991: **3**; 646–53.
6. Mulligan MS, Paulson JC, De Frees S, *et al.* Protective effects of oligosaccharides in P-selectin dependent lung injury. *Nature* 1993: **364**; 149–51.
7. Mayadas TN, Johnson RC, Rayburn H, Hynes RO, Wagner DD. Leukocyte rolling and extravasation are severely compromised in P selectin-deficient mice. *Cell* 1993: **74**; 541–54.
8. Hakomori S. New directions in cancer therapy based on aberrant expression of glycosphingolipids: anti-adhesion and ortho-signalling therapy. *Cancer Cells* 1991: **3**; 461–70.
9. Trail PA, Willner D, Lasch SJ, *et al.* Cure of xenografted human carcinomas by BR96–doxorubicin immunoconjugates. *Science* 1993: **261**; 212–15.
10. MacLean GD, Bowen-Yacyshyn MB, Samuel J, *et al.* Active immunization of human ovarian cancer patients against a common carcinoma (Thomsen–Friedenreich) determinant using a synthetic carbohydrate antigen. *J Immunother* 1992: **11**; 292–305.
11. Heitz P, Moser H, Staur JJ. Thyroid cancer. *Cancer* 1976: **37**; 2329–37.
12. Wynford-Thomas D, Williams ED. *Thyroid Tumours. Molecular Basis of Pathogenesis*. London: Churchill Livingstone, 1989.
13. Belloni PN, Nicolson LG. Role of the vascular endothelium in cancer metastasis. In: Simonescu N, Simonescu M, eds. *Endothelial Cell Dysfunctions*. New York, Plenum Press, 1992; 395–425.
14. Hakomori S. Le^x and related structures as adhesion molecules. *Histochem J* 1992: **24**; 771–6.
15. Lasky LA. Selectins: intepreters of cell-specific carbohydrate information during inflammation. *Science* 1992: **258**; 964–69.
16. Young WW, Portoukalian J, Hakomori S. Two monoclonal anticarbohydrate antibodies directed to glycosphingolipids with a lacto-N-glycosyl type II chain. *J Biol Chem* 1981: **256**; 10967–72.
17. Fukushi Y, Hakomori S, Nudelman E, Cochan N. Novel fucolipids accumulating in human adenocarcinoma. II. Selective isolation of hybridoma antibodies that differentially recognize mono-, di-, and trifucosylated type 2 chain. *J Biol Chem* 1984: **259**; 4681–5.
18. Abe K, McKibbin JM, Hakomori S. The monoclonal antibody directed to difucosylated type 2 chain. *J Biol Chem* 1983: **258**; 11793–7.
19. Bryne M, Thrane PS, Dabelsteen E. Loss of expression of blood group antigen H is associated with cellular invasion and spread of oral squamous cell carcinomas. *Cancer* 1991: **67**; 618–23.
20. Brodt P. Adhesion mechanisms in lymphatic metastasis. *Cancer Metastasis Rev* 1991: **10**; 23–32.
21. Brodt P. Tumor cell adhesion to frozen lymph node sections – an in-vitro correlate of lymphatic metastasis. *Clin Exp Metastasis* 1989: **7**; 343–52.
22. Bevilacqua MP, Nelson RM. Selectins. *J Clin Invest* 1993: **91**; 379–87.
23. Takada A, Ohmori K, Yoneda T, *et al.* Contribution of carbohydrate antigens sialyl Lewis A and sialyl Lewis X to adhesion of human cancer cells to vascular endothelium. *Cancer Res* 1993: **53**; 354–61.
24. Matsushita Y, Nakamori S, Seftor EA, Hendrix MJC, Irimura T. Human colon carcinoma cells with increased invasive capacity obtained by selection for sialyl-dimeric Le x antigen. *Exp Cell Res* 1991: **196**; 20–5.
25. Dejana E, Martin-Padura I, Lauri D, *et al.* Endothelial leukocyte adhesion molecule-1-dependent adhesion of colon carcinoma cells to vascular endothelium is inhibited by an antibody to Lewis fucosylated type I carbohydrate chain. *Lab Invest* 1992: **66**; 324–30.
26. Nakamori S, Kameyama M, Imaoka S, *et al.* Increased expression of sialyl Lewis x antigen correlates with poor survival in patients with colorectal carcinoma: clinicopathological and immunohistochemical study. *Cancer Res* 1993: **53**; 3632–7.
27. Vowden P, Lowe AD, Lennox SE, Bleechen MN. Thyroid blood group isoantigen expression: a parallel with ABH isoantigen expression in the distal colon. *Br J Cancer* 1986: **53**; 721–5.

28. Vierbuchen M, Schroeder S, Uhlenbruck G, Ortmann M, Fisher R. CA 50 and CA 19-9 antigen expression in normal, hyperplastic, and neoplastic thyroid tissue. *Lab Invest* 1989: **60**; 726–32.
29. Vierbuchen M, Larena A, Schroeder S, *et al.* Blood group antigen expression in medullary carcinoma of the thyroid. *Virch Arch B Cell Pathol* 1992: **62**; 79–88.
30. Singhal A, Hakomori S. Molecular changes in carbohydrate antigens associated with cancer. *BioEssays* 1990: **12**; 223–30.
31. Miyake M, Taki T, Hitomi S, Hakomori S. Correlation of expression of H/LeY/LeB antigens with survival in patients with carcinoma of the lung. *N Engl J Med* 1992: **37**; 14–18.
32. Dabelsteen E, Mandel U, Clausen H. Cell surface carbohydrates are markers of differentiation in human oral epithelium. *Crit Rev Oral Biol Med* 1991: **2**; 439–507.
33. Gillard BK, Thurmon LT, Marcus MD. Variable subcellular localization of glycosphingolipids. *Glycobiology* 1993: **3**; 57–67.
34. Susumu N, Kawakami H, Aoki D, Hirano H, Nozawa S. Subcellular localization of blood group Le^b carbohydrate antigen (MSN-1-reactive antigen) in endometrial cancer cells. *Cancer Res* 1993: **53**; 3643–8.
35. Limas C, Cutler B, Lange P. Ultrastructural localization of blood group antigen A in normal and neoplastic urothelium. *Histopathology* 1991: **18**; 1–10.

(Received 22 March 1994;
accepted in revised form 3 June 1994)