Histol Histopathol (1996) 11: 203-214

Histology and Histopathology

Invited Review

Histochemical demonstration and analysis of poly-N-acetyllactosamine structures in normal and malignant human tissues

N. Ito[1], M. Yokota[2], C. Nagaike[1], Y. Morimura[1], K. Hatake[1] and T. Matsunaga[2]

[1]Department of Legal Medicine and [2]Department of Otolaryngology, Nara Medical University, Kashihara Nara, Japan

Summary. Poly-N-acetyllactosaminyl structures carry a variety of physiologically and pathologically important carbohydrate antigens and are presumed to have essential roles in the process of cellular recognition, differentiation, malignant transformation and cancer metastasis. Monoclonal antibodies, lectins and endo-ß-galactosidase are useful histochemical tools for detecting and analyzing poly-N-acetyllactosamines in tissue sections. I (branched structure) and i (linear structure) antigens recognized by monoclonal antibodies have been shown to be differentiation antigens in mouse embryo and mouse and human teratocarcinoma cells as well as in human erythrocytes. They are also oncofoetal antigens and are expressed in carcinoma cells in several tissues and organs. Immobilized lectins specific to poly-N-acetyllactosamine structures have been successfully applied for fractioning glycoproteins with poly-N-acetyllactosamine, but histochemical use of these lectins has been restricted to some animal tissues. Among them, pokeweed mitogen agglutinin was used to detect branched poly-N-acetyllactosamine in normal and malignant human colon, demonstrating that it has a highly selective affinity for colorectal carcinomas. *Griffonia simplicifolia* agglutinin-II staining following endo-ß-galactosidase digestion procedure revealed the presence of poly-N-acetyllactosamine structures with or without blood group-specificities in several normal human tissues. By using this procedure, it was demonstrated that the blood group-related antigens oncofoetally expressed in thyroid carcinoma cells are carried by poly-N-acetyllactosamines containing a domain susceptible to the enzyme digestion. Staining with lectins specific to poly-N-acetyllactosamine in combination with endo-ß-galactosidase digestion demonstrated that poly-N-acetyllactosaminyl structures ubiquitously and consistently produced in thyroid papillary carcinomas are highly heterogeneous in their chain length and branching status and quite different from those produced in other thyroid neoplasms. Staining with monoclonal antibodies or lectins combined with endo-ß-galactosidase digestion procedures have been proven to be powerful tools for localizing and analyzing different types of poly-N-acetyllactosamine structures in normal and malignant tissues.

Key words: Poly-N-acetyllactosamine, Blood group antigen, Monoclonal antibody, Lectin, Endo-ß-galactosidase

Offprint requests to: Dr. Nobuaki Ito, Ph.D., Department of Legal Medicine, Nara Medical University, Kashihara Nara 634, Japan

Introduction

The repeating disaccharides (Galß1-4GlcNAcß1-3) or poly-N-acetyllactosamine sequences were initially discovered in asparagine-linked (N-linked) oligosaccharides of glycoproteins present on human erythrocytes (band 3 and band 4.5) and they have been proven to contain the majority of ABH blood group antigens in erythrocytes (Fukuda, 1985, 1994; Laine and Rush, 1988). After this, poly-N-acetyllactosaminyl structures have been found in glycosphingolipids (Hakomori, 1981) and serine- or threonine-linked (O-linked) as well as N-linked oligosaccharides in a variety of animal cell glycoproteins (Feizi, 1981; Feizi and Childs, 1987; Fukuda, 1985, 1994; Hanisch et al., 1989; Maemura and Fukuda, 1992).

Recently, much attention has been paid to these complex carbohydrate structures since they carry several different kinds of carbohydrate antigens which are implicated in selectin-mediated cell-cell interactions (Cummings and Smith, 1992; Feizi, 1992; Fukuda, 1994; Moore et al., 1994), sperm-oocyte interaction (Kinloch et al., 1991), and serve as the binding sites for some bacteria to host tissues (Loomes et al., 1984; Friedman et al., 1985; Loveless and Feizi, 1989; Liukkonen et al., 1992; Loveless et al., 1992). Recent studies have also demonstrated that dramatic changes occur in the size and amounts of poly-N-acetyllactosamine during differentiation and malignant transformation of

embryonic cells (Pennington et al., 1985; Muramatsu, 1988; Muramatsu and Muramatsu, 1990; Hefernan et al., 1993 Spillman and Finne, 1994), haematopoietic cells (Fukuda, 1985; Fukuda et al., 1986; Irimura et al., 1987; Lee et al., 1990; Rabinowitz and Gordon, 1991) and certain cancer cell lines (Yamashita et al., 1984; Hubbard, 1987; Saitoh et al., 1992; Bierhuizen et al., 1994). It has been further demonstrated that the presence of poly-N-acetyllactosamine structures appears to be essential for metastatic potential of lymphoid tumor cell line (Laferte and Dennis, 1989) and sublines of human colon carcinoma (Saitoh et al., 1992). These facts may indicate that poly-N-acetyllactosamines play many important roles in the process of cellular recognition, differentiation and malignant transformation. It is indispensable for us to know the precise distribution of different types of poly-N-acetyllactosamines both in normal and malignant tissues in order to promote a better understanding on the roles of these complex carbohydrate structures in physiological and pathological processes.

At present, three kinds of histochemical tools are available for detecting and analyzing poly-N-acetyllactosamine in tissue sections, i.e., monoclonal antibodies, lectins and endo-ß-galactosidase. In this article, we review the findings obtained by the studies employing these reagents and then focus on our recent approach to analyze poly-N-acetyllactosamines with blood group-related antigens in human thyroid neoplasms by using lectins and endo-ß-galactosidase digestion.

Monoclonal antibodies specific to poly-N-acetyllactosamine and their histochemical application

Human autoantibodies and hybridoma antibodies with anti-I and i activities are known to recognize branched and linear poly-N-acetyllactosaminyl structures, respectively (Feizi; 1981; Hakomori, 1981; Fukuda, 1985, 1994). Specificities of several antibodies have been critically examined and some of them have been used for histochemical studies (Table 1). The Ii antigens represent the adult(I) and foetal(i) human erythrocyte antigens and the conversion from i to I antigens takes place during development within a year after birth. It is interesting to investigate whether these antigens are also the differentiation antigens in other human and animal tissues as in erythrocytes.

In fact, the Ii antigens have been shown to rank as «stage-specific» antigens in early differentiation of mouse embryos *in vivo* (Pennington et al., 1985; Muramatsu, 1988) and mouse and human teratocarcinomas *in vitro* (Kapadia et al., 1981a; Childs et al., 1983). In addition, both I and sialyl I antigens are specific markers for the differentiated type cells in each stage of development of the human lung, while Le^x and related embryonic antigens are specific to the immature bud cells in early stage (Itai et al., 1990). Furthermore, linear and branched poly-N-acetyllactosaminyl

Table 1. Human autoantibodies and monoclonal antibodies against poly-N-acetyllactosamine.

ANTIBODY	SPECIFICITY	REFERENCE
Anti-i (Ma)	Galß1-4GlcNAcß1-6Gal	Gooi et al., 1984
Anti-I (Step)	Galß1-4GlcNAcß1-3Gal	Gooi et al., 1984
Anti-i (Den)	$(Galß1\text{-}4GlcNAcß1\text{-})_3$	Feizi et al., 1979 Gooi et al., 1984
MH21-134	$(Galß1\text{-}4GlcNAcß1)_2\text{-}3Galß1\text{-}R$	Miyake et al., 1989
ACFH-18	Fucosylated poly-N-acetyllactosamine	Nudelman et al., 1988
FW6	Fucosylated poly-N-acetyllactosamine	Hanisch et al., 1993 Schwonzen et al., 1992

Gal: D-Galactose; GlcNAc: N-acetyl-D-glucoosamine.

structures have been demonstrated in colonic and hepatocellular carcinoma cells (Miyake et al., 1989), mucous-producing adenocarcinomas of the human lung (Hirohashi et al., 1984) and gastric carcinomas (Picard et al., 1978; Kapadia et al., 1981b) by immunohistochemical studies using the human monoclonal antibody against linear poly-N-acetyllactosamine (MH21-134, see Table 1) or anti-i and anti-I autoantibodies. Dohi et al. (1990) detected fucosylated polylactosamine in gastric carcinoma cells and proliferating zone of normal fundic gland by a monoclonal antibody ACFH-8 which was established by immunizing mice with human gastric cancer cell line MKN-74 (Nudelman et al., 1988) (see Table 1). Similar mouse monoclonal antibody against fucosylated polylactosamine has been reported and found to react with foetal colon and colon carcinoma (Hanisch et al., 1993) (see Table 1). Since Ii and related antigens have not been detected in normal counterpart cells, these antigens are cancer associated oncofoetal antigens. Some of the antibodies against these antigens, therefore, may be valuable tools in cancer diagnosis or cancer treatment.

As described in the following section, poly-N-acetyllactosaminyl structures have been shown to be present in several normal human tissues. Although some information on normal animal tissues is available (Römer et al, 1979), comprehensive studies have not yet been carried out on the distribution of poly-N-acetyllactosamines recognized by anti-Ii antibodies in normal human tissues. Thus, our knowledge on the distribution of Ii-related antigens in normal tissues is fairly restricted.

In normal rat and human kidney, poly-N-acetyllactosamines expressed by epithelia of Henle's loop and collecting ducts were selectively recognized by anti-Ii antibodies (Lenhard et al., 1978; Raghunath et al., 1994). Consistent with the observation by Itai et al. (1990), Ii antigens and their sialylated forms were detected in ciliated cells of normal human bronchial epithelia by light and electron microscopic studies (Loveless and Feizi, 1989; Loveless et al., 1992). These antigens are major host cell receptors for the human pathogen

Micoplasma pneumoniae (Loomes et al., 1984). Since Ii antigens are the backbone precursor structures of blood group ABH antigens in erythrocytes (Fukuda, 1985, 1994), they are expected to express in tissues more preferentially from nonsecretors than secretors. As expected, in human gastric mucosae and cervic glands, anti-I or -i antibodies reacted preferentially with the tissues from nonsecretor individuals (Kapadia et al., 1981a,b; Griffin and Wells, 1993).

Although monoclonal antibodies are the most reliable for demonstrating definite structures of poly-N-acetyllactosaminyl structures, their limited availability and narrow or strict specificities make it difficult to carry out comprehensive and extensive studies on the distribution of these structures. Furthermore, monoclonal antibodies cannot usually recognize the inner backbone structures if they are capped with blood group-related antigens at their non-reducing termini.

Lectins specific to poly-N-acetyllactosamine and their histochemical application

Among the lectins with the nominal specificities for GlcNAc, many of them exhibit much stronger specificities for oligosaccharide of chitin (a homopolymer of GlcNAc in (ß1-4) linkage) derivatives (Goldstein and Poretz, 1986). Since these oligosaccharides and chitin are found in the exoskeletons of insects and crustaceans but not in the constituents of mammalian tissues, including humans, binding of these lectins in tissue sections may not be due to these substances, but are presumed to be linear or branched poly-N-acetyllactosaminyl structures. In fact, some lectins such as DSA, LEA and PWM have been shown to interact strongly with poly-N-acetyllactosaminyl structures (Irimura and Nicolson, 1983; Crowley et al., 1984; Merkle and Cummings, 1987; Yamashita et al., 1987; Zhu and Laine, 1989).

Recent biochemical studies have shown that immobilized DSA, LEA and PWM are useful tools for fractioning glycopeptide with poly-N-acetyllactosamines. Thus, DSA is known to interact with one or two short poly-N-acetyllactosamine side chains, whereas LEA bound to three or more longer poly-N-acetyllactosamine sequences (Crowley et al., 1984; Merkle and Cummings, 1987; Yamashita et al., 1987; Lee et al., 1990). On the other hand, PWM was found to recognize branched but not linear poly-N-acetyllactosamine (Irimura and Nicolson, 1983; Dutt et al., 1988; Muramatsu and Muramatsu, 1990). Hence, specificities of LEA and PWM may well correspond to those of anti-i and -I antibodies, respectively. Immobilized WGA also binds and retains poly-N-acetyllactosamine, but the interaction is complex, requiring fucose residues not directly involved in lectin-binding, and being independent of or even hindered by adjacent sialic acid residues (Gallagher et al., 1985; Ivatt et al., 1986; Rabinowitz and Gordon, 1991). Since Suc-WGA binds glycoconjugate containing GlcNAc but does not bind glycoconjugate containing sialic acids (Monsigny et al., 1980), its interaction with poly-N-acetyllactosamine may not be affected by proximal sialic acid. Although the binding ability of other GlcNAc specific lectins such as STA with poly-N-acetyllactosamine has not yet been examined, it seems very probable that they would also recognize the sequences since they are similar to WGA in their ability to bind derivatives of chitin oligomers (Goldstein and Poretz, 1986).

Table 2. Sources and specificities of lectins used for localizing poly-N-acetyllactosaminyl structures in tissue sections.

SISTEMATIC NAME (common name)	ACRONYM	SPECIFICITY
Datura stramonium	DSA	(Galß1-4GlcNAcß1-3)$_{1-2}$ | ß1-6 Man | ß1-2 Galß1-4GlcNAc
Lycopersicon esculentum (Tomato)	LEA	(Galß1-4GlcNAcß1-3)$_n$
Phytolacca americana (Pokeweed)	PWM	[Galß1-4GlcNAcß1-3 | ß1-6 Galß1-4GlcNAc]$_n$
Solanum tuberosum (potato)	STA	-[a]
Triticum vulgare (Wheat germ)	WGA	-[a]
Succinyl *Triticum vulgare* (Wheat germ)	Suc-WGA	-[a]

Gal: D-galactose; GlcNAc: N-acetyl-D-glucosamine; Man: D- mannose; [a]: not identified.

Recently, some of these reagents have been used for histochemical localization of poly-N-acetyllactosamine structures in different animal tissues (Egea et al., 1989; Callaghan et al., 1990; Hughes and Rudland, 1990a,b; Evans et al., 1994). However, only a few reports have appeared on histochemical study with these lectins in human tissues (Aoki et al., 1993). Accordingly, in this case too, comprehensive histochemical studies should be carried out on the binding ability of these lectins in normal and malignant human tissues. Table 2 illustrates the lectins used for localizing poly-N-acetyllactosaminyl structures in tissue sections and their specificities.

Callaghan et al. (1990) demonstrated that LEA and STA strongly react with intracellular membrane network of gastric parietal cells of rat, dog and pig by light and electron microscope observation. They further showed that membrane glycoprotein recognized by these lectins is the subunit of the gastric H^+/K^+-ATP ase (proton pump). On the other hand, Acarin et al. (1994) showed that LEA selectively bound poly-N-acetyllactosamine in ameboid and ramified microglial cells in rat brain. Hughes and Rudland (1990a,b) found that PWM was useful for identification of normal, developing and neoplastic myoepithelial cells in rat mammary glands. DSA has also been shown to be useful for demonstrating poly-N-acetyllactosaminyl structures in light and electron microscope studies (Egea et al., 1989) and has

been applied for demonstrating poly-N-acetyllactosamine carried by lysosomal membrane glycoproteins, h-lamp-1, h-lamp-2 (Carlsson et al., 1988). Recently, Aoki et al. (1993) demonstrated that PWM has a highly selective affinity for human colorectal carcinomas, suggesting that lectins are also useful for detecting poly-N-acetyllactosamine expressed in malignant human tissues. Other lectins, such as WGA and STA were frequently and routinely used for demonstrating aberrant glycosylation during malignant transformation of many tissues. However, their binding sites were usually explained by the presence of GlcNAc or chitin oligomers. We think that poly-N-acetyllactosamines are much more plausible candidates for the binding sites of these lectins. If the lectin binding sites are properly appreciated, our knowledge on the distribution of these complex carbohydrate structures may be amplified.

Since the specificity of lectins is broad and is not so strict, the possibility that the binding sites of lectins are not ascribed to poly-N-acetyllactosamine structures cannot be neglected. Studies combined with exo- and endo-glycosidase digestion procedures would be required for determining the structures recognized by these lectins in histochemical systems.

Endo-ß-galactosidase and its histochemical application

Endo-ß-galactosidase from *Escherichia freundii* attacks the internal ß-galactoside linkage of the linear lactosamine sequence and catalyzes the following reaction (Fukuda and Matsumura, 1976; Fukuda et al., 1978; Maley et al., 1989):

$$\text{R-GlcNAcß1-3Galß1-4GlcNAcß1-R'} + H_2O \longrightarrow \text{R-GlcNAcß1-3Gal} + \text{GlcNAcß1R'}$$

The enzyme cannot hydrolyze this linkage when Gal residues are further substituted at position C-6 by GlcNAc to form a branch point (Scudder et al., 1984). Substitution of Gal by sulphate, and fucosylation of residues in close proximity to the susceptible linkage also inhibit the enzyme's action (Fukuda and Matsumura, 1976; Kannagi et al., 1982; Scudder et al., 1984).

The enzyme has been used for characterization of poly-N-acetyllactosamine structures from various normal and malignant cells in biochemical and physicochemical studies (Tsay et al., 1975; Fukuda et al., 1978, 1979; Fukuda et al., 1984a,b; Fukuda, 1994; Wang et al., 1991). Since the lectin *Griffonia simplicifolia* agglutinin-II (GSA-II) specifically reacts with terminal non-reducing GlcNAc residues in tissue sections (Ebisu et al., 1978; Ito and Hirota, 1992), it is to be expected that the distribution of R-GlcNAcß1-3Galß1-4GlcNAcß1-R' structure can be demonstrated in tissue section by using GSA-II staining following endo-ß-galactosidase digestion.

Ito et al. (1994a) successfully introduced endo-ß-galactosidase (from *Escherichia freundii*) -GSA-II staining procedure into carbohydrate histochemistry to demonstrate poly-N-acetyllactosamine structures in formalin-fixed and paraffin-embedded normal human tissues. Endo-ß-galactosidase digestion reveals GSA-II reactivity in the following tissues and cells (Table 3): pancreatic acinar cells (Fig. 1a,b); gastric surface mucosae; duct cells and mucous cells of salivary glands and tracheal glands; surface epithelium of trachea; goblet cells of large intestine; columnar epithelium of uterine cervical glands; distal and collecting tubules of kidney; certain cells of anterior lobe and colloid of middle lobe of pituitary glands; epithelial reticular cells and Hassall's corpuscles of thymus; and Kupffer cells of liver. Endo-ß-galactosidase from *Bacteroides fragilis* has almost the same specificities for oligosaccharides as that for *Escherichia freundii* (Scudder et al., 1984) and similar histochemical results were obtained by using this enzyme (Ito et al., unpublished observation).

Consistent with the results obtained by using monoclonal anti Ii antibodies (Kapadia et al., 1981b), endo-ß-galactosidase digestion revealed the presence of

Table 3. Effects of endo-ß-galactosidase (E-ß-G) digestion and sequential fucosidase-E-ß-G digestion on GSA-II staining in human tissues.

		DIGESTION WITH E-ß-G		DIGESTION WITH FUCOSIDASE AND E-ß-G
Pancreatic acinar cells		(E)	+	++
Duct cells of submandibular and parotid glands		(E)	+	++
Epithelial cells of trachea		(E)	+	+
Anterior pituitary cells		(N)	+	+
Colloid of middle lobe of pituitary		(E)	+	+
Hassall's corpuscles of thymus		(E)	+	+
Epithelial reticular cells of thymus		(E)	+	+
Distal and collecting tubule of kidney		(E)	+	+
Kupffer cells of liver		(N)	+	+
Epithelial cells of cervic gland		(E)	+	+
Goblet cells of large intestine		(N)	+	+
Gastric surface mucosae	(S)	(E)	-	+
	(NS)	(N)	+	+
Mucous cells of salivary and tracheal glands	(S)	(E)	-	-
	(Le(a+b-))(NS)	(N)	-	-
	(Le(a-b-)) (NS)	(N)	+	+

S: secretor; NS: non-secretor; -: no staining; +: staining; ++: staining enhanced by prior fucosidase digestion; E: blood group antigens are expressed in these tissue sites (Ito and Hirota, 1992); N: blood group antigen are weakly or not at all expressed in these tissue sites (Ito and Hirota, 1992).

enzyme-susceptible poly-N-acetyllactosamine in gastric surface mucosae from non-secretor individuals, but not secretor individuals. Furthermore, Ito et al. (1994a) demonstrated the presence of enzyme-susceptible poly-N-acetyllactosamine in mucous cells of salivary and tracheal glands from Le(a-b-) non-secretor individuals but not in Le(a+b-) non-secretors or Le(a-b+) secretor individuals. On the other hand, in pancreatic acinar cells and duct cells of salivary glands from foetuses and newborn infants, prior fucosidase digestion markedly enhanced the GSA-II reactivity elicited by endo-ß-galactosidase digestion. Prior fucosidase digestion was also a prerequisite for demonstrating poly-N-acetyllactosamine by endo-ß-galactosidase digestion in gastric surface mucosae from secretor individuals. Endo-ß-galactosidase digestion eliminated the ABH antigens in vascular endothelial cells and Le^a antigen in pancreatic duct cells, although GSA-II reactivity did not appear in these tissue sites. Thus, it is confirmed that these cells and tissue produce poly-N-acetyllactosamine structures which constitute the inner backbone structures of blood group-related antigens.

As described here, endo-ß-galactosidase is a useful reagent for analyzing different types of poly-N-acetyllactosamines with or without blood group specificities, and accordingly, a much more versatile tool than monoclonal antibodies and lectins since the use of this enzyme provides information about distribution of not only the linear domain of poly-N-acetyllactosamine structures but also the antigens carried by these complex backbone structures. The use of this enzyme is expected to provide valuable information as to the aberrant glycosylation of carbohydrate chains in malignant cells in histochemical systems.

Poly-N-acetyllactosamine and blood group-related antigens in thyroid neoplasms

In normal thyroid glands, blood group ABH and related antigens are not expressed in any cells except for vascular endothelial cells (Ito and Hirota, 1992). These antigens, however, have been known to appear in malignant thyroid tissues, especially in papillary carcinomas in high frequency (Vowden et al., 1986; Vierbuchen et al., 1989, 1992). Since these antigens have been shown to be expressed in thyroid epithelial cell surfaces in the earliest developmental stage and disappear by the time the final adult histological structure is observed (Szulman, 1964), such phenomena have been regarded as oncofoetal expression of the

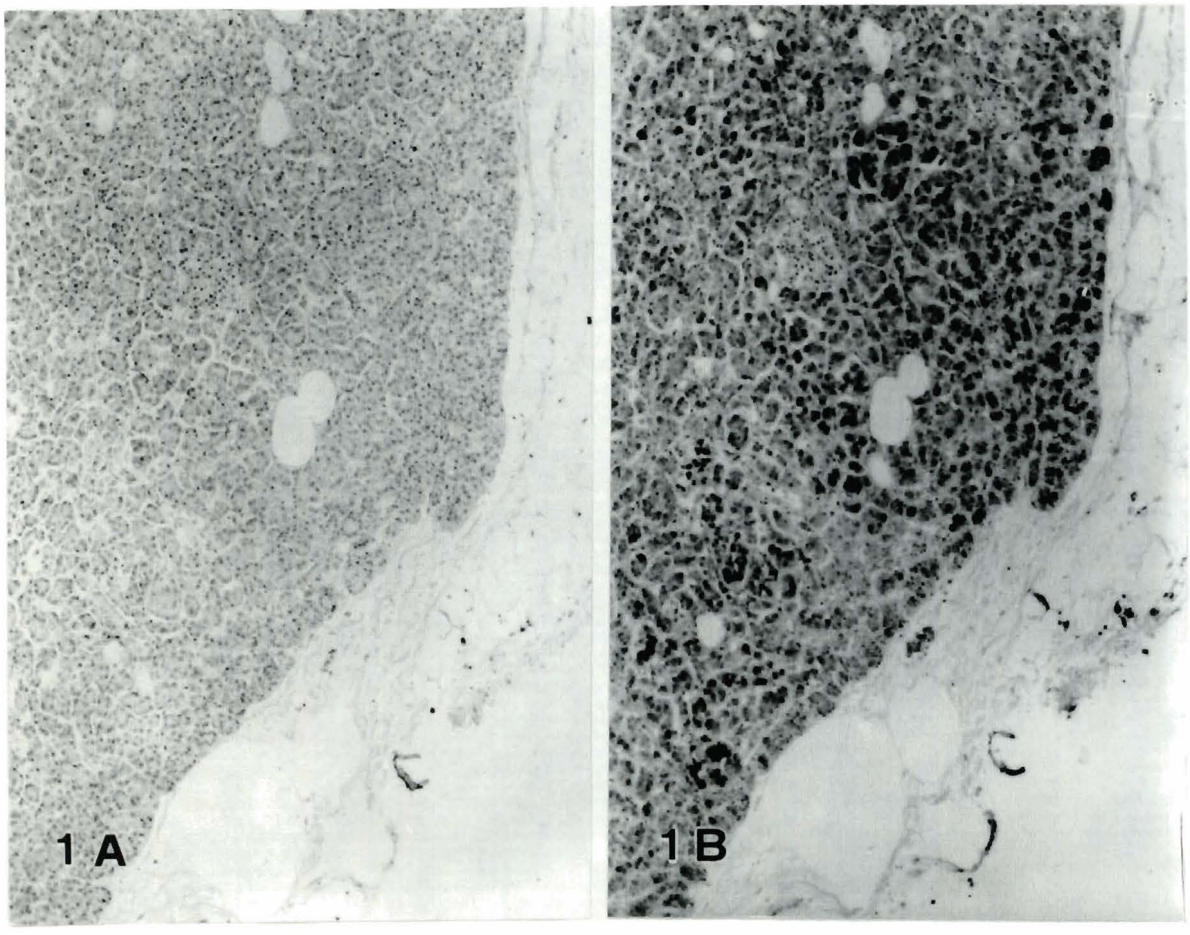

Fig. 1. Sections of pancreas from a blood group AB secretor individual stained with GSA-II before (**A**) and after (**B**) endo-ß-galactosidase digestion. Without enzyme digestion, no reactivity with GSA-II is observed, whereas marked reaction with GSA-II is seen in nearly all the acinar cells after enzyme digestion. Sections were counterstained with Haematoxylin. From Ito et al. (1994a). x 100

blood group antigens (Vowden et al., 1986; Lloyd, 1987; Vierbuchen et al., 1989, 1992; Ito and Hirota, 1992). Similar oncofoetal expression of the blood group related-antigens has been reported in other organs and tissues such as hepatic carcinomas and adenocarcinomas of distal colon (Lloyd, 1987; Ito and Hirota, 1992). However, the mechanism of oncofoetal expression of the blood group-related antigens has not yet been fully understood.

Ito et al. (1994b) demonstrated that in papillary thyroid carcinomas, re-expressed, blood group-related antigens such as ABH, Lewis a, Lewis b, sialylated Lewis a (CA 19-9), and sialylated precursor type 1 chain are all carried by poly-N-acetyllactosamines which are susceptible to endo-ß-galactosidase digestion (Fig. 2a,b). Along with the disappearance or reduction of these antigens, GSA-II reactivity appeared ubiquitously and consistently in corresponding luminal cell surfaces and cytoplasm from all the individuals examined following enzyme digestion (Ito et al., 1994b) (Fig. 3a,b) (Table 4). Therefore, the most important and basic mechanism of neo- or onco-foetal expression of the blood group antigens in thyroid papillary carcinoma is due to the neo-expression of poly-N-acetyllactosamine structure, since the structures are common and direct precursor of the blood group-related antigens in cancer cells. This suggested that neoplastic transformation and tumor progression is critically and intimately related to induction or derepression of the extension and/or branching enzymes of lactosamine structures as demonstrated in certain cancer cell lines (Yamashita et al., 1984; Fukuda, 1985, 1994; Hubbard, 1987; Lee et al., 1990).

Yokota et al. (1995) found that poly-N-acetyl-lactosamines susceptible to endo-ß-galactosidase digestion are also backbone carrier structures of blood group-related antigens expressed in thyroid neoplasms other than papillary carcinomas, such as adenomas, follicular carcinomas and anaplastic carcinoma. Thus, the expression of blood group-related antigens may be regulated through the synthesis of backbone, poly-N-acetyllactosamine structures rather than terminal glycosylation in these thyroid neoplasms, as in papillary carcinomas. However, GSA-II reactivity did not appear in these thyroid neoplasms as in the case of vascular endothelial cells and pancreatic duct cells (Ito et al., unpublished observation) (Table 4), suggesting that the structures of poly-N-acetyllactosamine produced in papillary carcinomas are quite different from those produced in other types of thyroid neoplasms. It is

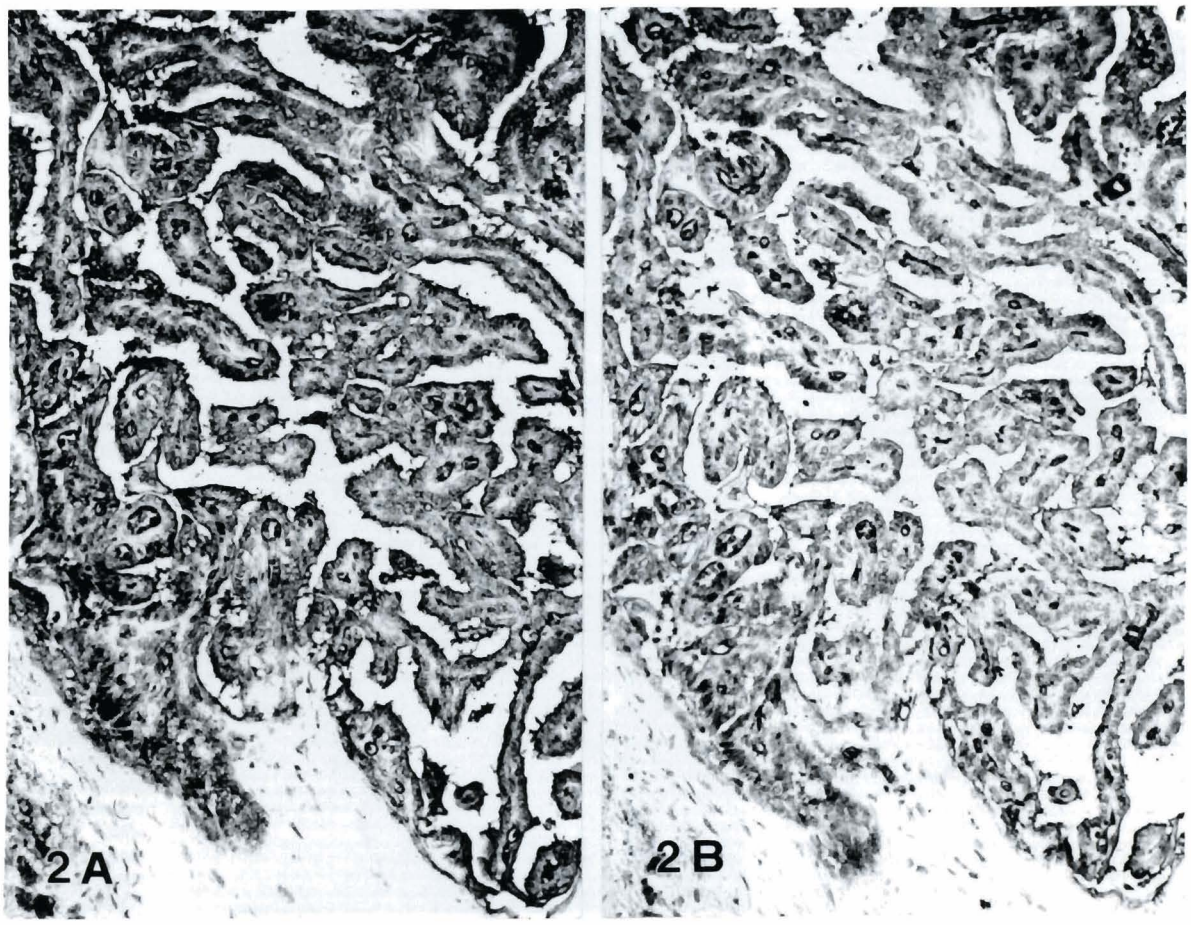

Fig. 2. Sections of thyroid papillary carcinomas from a blood group A individual stained with anti-A antibody before (**A**) and after (**B**) endo-ß-galactosidase digestion. Anti-A antibody reacts weakly with cytoplasm and strongly with luminal surfaces of cancer cells. Following enzyme digestion, elimination or marked reduction of reactivity with the antibody is seen in these tissue sites. Sections were counterstained with Haematoxylin. x 200

presumed that the blood group-related antigens are built on at least two different species of poly-N-acetyllactosamine, i.e., one containing GlcNAc residues, which are recognized by labelled GSA-II after exposure by enzyme digestion, and the other containing residues to which GSA-II for an unknown reason is not accessible even after enzyme digestion.

More recently, Ito et al. (1995) attempted to analyze in detail the backbone poly-N-acetyllactosamine structures found in thyroid neoplasms by using labelled lectins exhibiting specificities for different poly-N-acetyllactosamine structures. The results further confirmed that poly-N-acetyllactosamine species found in papillary carcinomas are quite different from those in other types of thyroid neoplasms and at least three different types of poly-N-acetyllactosamine, that is, linear unbranched short (DSA reactive and endo-ß-galactosidase resistant) and long sequences (LEA reactive and endo-ß-galactosidase labile) and highly branched (PWM reactive and endo-ß-galactosidase labile) ones are preferentially produced in papillary carcinomas (Table 4). The production of such highly heterogeneous structures of poly-N-acetyllactosamine may be related to the great tendency of papillary

Table 4. Reactivity of thyroid neoplasms with the antibodies against blood group ABH antigens and lectins.

	No. OF REACTIVE CELLS, No. (%) OF POSITIVE SPECIMENS		
	Papillary carcinoma (n=20)	Adenoma (n=7)	Follicular carcinoma (n=8)
Antibodies against ABH antigen	+1~ +4, 20 (100)	+1~ +2, 2 (30)	+1~ +4, 5 (63)
DSA	+3~ +4, 20 (100)	+1~ +2, 3 (42)	+1, 3 (38)
LEA	+1~ +4, 16 (80)	-, 0 (0)	+1, 2 (25)
PWM	+1~ +4, 20 (100)	+1, 1 (13)	+1, 2 (25)
GSA-II	+1, 1 (5)	-, 0 (0)	-, 0 (0)
E-ß-G - GSA-II	+3~ +4, 20 (100)	-, 0 (0)	+1, 1 (13)

+4: >90% cells bind; +3: 50-90% cells bind; +2: 10-50% cells bind; +1: <10% cell bind; -: no cells bind; E-ß-G: endo-ß-galactosidase; n: number of specimes examined.

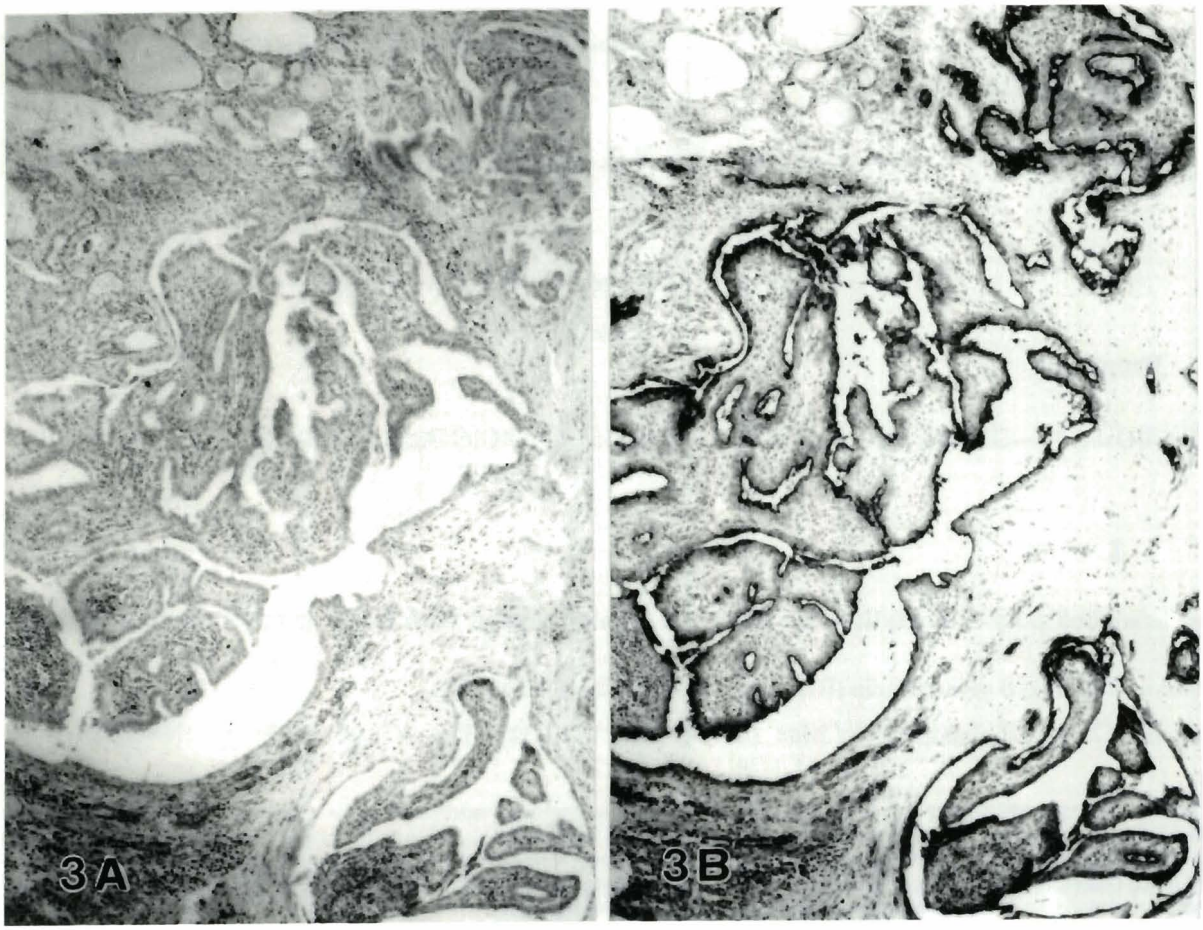

Fig. 3. Sections of papillary carcinomas from a blood group A individual stained with GSA-II before (**A**) and after (**B**) endo-ß-galactosidase digestion. Following enzyme digestion, nearly all the cells and cell surfaces are strongly stained with GSA-II, while without enzyme digestion, no reactivity with GSA-II is seen. Sections were counter-stained with haematoxylin. x 100

carcinomas to invade lymph vessels and regional lymph node (Ljungberg, 1992). In fact, the presence of poly-N-acetyllactosamine has been shown to be essential for metastatic potential of lymphoid tumor cell line (Laferte and Dennis, 1989) and sublines of human colon carcinoma (Saitoh et al., 1992). At present, structural features of poly-N-acetyllactosamine with the blood group antigens in other types of neoplasms are not known except that they are susceptible to endo-ß-galactosidase digestion. Fig. 4 illustrates the proposed main biosynthetic pathways of poly-N-acetyllactosamine structures in papillary carcinomas.

By using endo-ß-galactosidase-GSA-II staining or lectin staining procedures, it has been possible to analyze the mechanism of oncofoetal expression of blood group-related antigens in human thyroid neoplasms, demonstrating that the synthesis of poly-N-acetyllactosamine is one of the most critical and invaluable steps of neoplastic transformation leading to the progression of different types of neoplasia. Since similar oncofoetal expression of the blood group-related antigens has been reported in hepatic carcinomas and adenocarcinomas of the distal colon (Lloyd, 1987; Ito and Hirota, 1992), it would be interesting to investigate whether the blood group-related antigens found in these neoplasms are also carried by poly-N-acetyllactosamine structures.

Conclusion

As described in this article, monoclonal antibodies, lectins and endo-ß-galactosidase are useful histochemical reagents for detecting and analyzing poly-N-acetyllactosamine in tissue sections. These reagents have their own advantages and defects as histochemical tools. That is, specificity of monoclonal antibodies is most reliable, but their restricted availability and narrow or strict specificity prevents their extensive and conventional use for histochemical studies. Although the specificity of lectins is broad and is not usually well

Initial core branching

GlcNAc β 1→6R_1 or R_2

↓

Gal β 1→4GlcNAc β 1→6R_1 or R_2 ← DSA

Chain extention

↓ Gal β 1→4GlcNAc β 1→3Gal β 1→4GlcNAc β 1→6R_1 or R_2 ← DSA

↓ (Gal β 1→4GlcNAc β 1→3)$_n$Gal β 1→4GlcNAc β 1→6R_1 or R_2 ← LEA

($n \geqq 3$)

Branching

(Gal β 1→4[Gal β 1→4GlcNAc β 1→6]GlcNAc β 1→3)$_n$Gal β 1→4GlcNAc β 1→6R_1 or R_2 ← PWM

↓

Linear and branched poly-N-acetyllactosamines are finally capped with blood group-related antigens.

R_1; Core structure of O-linked glycoprotein ([Gal β 1→3]GalNAc α 1→Ser/Thr)

R_2; Core structure of N-linked glycoprotein (Man α 1→6[Man α 1→3]Man β 1→4GlcNAc β 1→4GlcNAc β 1→Asn)

Fig. 4. Proposed main biosynthetic pathways of poly-N-acetyllactosamine structures in papillary carcinomas of the human thyroid glands. DSA reacts with one or two short poly-N-acetyllactosamine side chains whereas LEA binds to three or more long poly-N-acetyllactosamine sequences. On the other hand, PWM recognizes branched but not linear poly-N-acetyllactosamine. Linear long or highly branched poly-N-acetyllactosamines are finally capped with blood group-related antigens. Since these poly-N-acetyllactosaminyl structures are susceptible to endo-ß-galactosidase digestion, LEA and PWM binding sites and blood group-related antigens are eliminated by the enzyme digestion.

defined, commercial availability of lectins accelerates their use as convenient histochemical tools.

Endo-ß-galactosidase can effectively hydrolyze a vast variety of poly-N-acetyllactosamine structures (Fukuda, 1994). However, structural characteristics of poly-N-acetyllactosamines cannot be deduced by enzyme digestion, except for the fact that they contain a linear domain susceptible to the enzyme. In addition, the enzyme cannot hydrolyze highly branched and highly decorated structures. To overcome the limitation suffered from these defects, the combined use of different types of reagents, especially monoclonal antibodies or lectins with endo-ß-galactosidase digestion should be preferable. This enables us to differentiate and subdivide poly-N-acetyllactosaminyl structures as well as to determine peripheral antigenic determinants according to their affinity for monoclonal antibodies or lectins and susceptibility to the enzyme. In addition, if oligosaccharides released from poly-N-acetyllactosamine can be directly analyzed by the reliable biochemical methods such as high-performance liquid chromatography (HPLC), we can deduce the whole sequences of poly-N-acetyllactosamine in tissue sections. Application of HPLC has already been carried out by Ito et al. (1993) to estimate Gal residues released by α-galactosidase digestion in tissue sections.

Although our knowledge about the structures and functions of poly-N-acetyllactosamine in human tissues is still fragmentary and far from satisfactory, series of our studies provide some clues to a better understanding of these exciting problems at the histo- and cytochemical levels. In particular, successful introduction of endo-ß-galactosidase in carbohydrate histochemistry has been proven to be prominently important and will make it possible to realize much more systematic and consistent analysis of such complex carbohydrate chains in histochemical systems. The use of monoclonal antibodies and lectins specific to core portion of N- or O-linked glycoproteins is also essential for such purposes. Through these studies, it may be established that the regulation of cell surface antigenic systems is performed through the synthesis of backbone, poly-N-acetyllactosamine structures as well as terminal glycosylation. It is hoped that the methods developed by us will contribute to further progress in carbohydrate histochemistry and glycobiology.

References

Acarin L, Vela J.M., González B. and Castellano B. (1994). Demonstration of poly-N-acetyl lactosamine residues in ameboid and ramified microglial cells in rat brain by tomato lectin binding. J. Histochem. Cytochem. 42, 1033-1041.

Aoki T., Kawano J., Oinuma T., Haraguchi Y., Eto T. and Suganuma T. (1993). Human colorectal carcinoma-specific glycoconjugates detected by pokeweed mitogen lectin. J. Histochem. Cytochem. 41, 1321-1330.

Bierhuizen M.F.A., Maemura K. and Fukuda N. (1994). Expression of a differentiation antigen and poly-N-acetyllactosaminiyl O-glycans directed by a cloned core 2 ß1, 6-N-acetylglucosaminyltransferase. J. Biol. Chem. 269, 4473-4479.

Callaghan J.M., Toh B.-H, Pettitt J.M., Humphris D.C. and Gleeson P.A. (1990). Poly-N-acetyllactosamine-specific tomato lectin interacts with gastric parietal cells. Identification of a tomato-lectin binding 60-90x10^3 Mr membrane glycoprotein of tubulovesicles. J. Cell Sci. 95, 536-576.

Carlsson S.R., Roth J., Piller F. and Fukuda M. (1988). Isolation and characterization of human lysosomal membrane glycoproteins, h-lamp-1 and h-lamp2. J. Biol. Chem. 263, 18911-18919.

Chids R.A., Pennington J., Uemura K., Scudder P., Goodfellow P.N., Evans M.J. and Feizi T. (1983). High-molecular-weight glycoproteins are the major carriers of the carbohydrate differentiation antigens I, i and SSEA-1 of mouse teratocarcinoma cells. Biochem. J. 215, 491-503.

Crowley J.F., Goldstein I.J., Arnap J. and Lonngren J. (1984). Carbohydrate binding studies on the lectin from *Datura stramonium* seeds. Arch. Biochem. Biophys. 231, 524-533.

Cummings R.D. and Smith D.F. (1992). The selectin family of carbohydrate-binding proteins: structure and importance of carbohydrate ligands for cell adhesion. BioEssays 14, 849-856.

Dohi T., Abe K., Ohshiba S., Yamaguchi K. and Oshima M. (1990). Immunohistochemical and biochemical detection of fucosylated polylactosamine antigen with monoclonal antibody ACFH18 in gastric cancer and normal gastric mucosa. Jpn. J. Cancer Res. 81, 676-681.

Dutt A., Tang J-P. and Carson D.D. (1988). Estrogen preferentially stimulates lactosaminoglycan-containing oligosaccharide synthesis in mouse uteri. J. Biol. Chem. 263, 2270-2279.

Ebisu S., Iyer S. and Goldstein I.J. (1978). Equilibrium dialysis and carbohydrate-binding studies on the 2-acetimido-2-deoxy-D-glucopyranosyl-binding lectin from *Bandeiraea simplicifolia* seeds. Carbohydr. Res. 61, 129-138.

Egea G., Goldstein I.J. and Roth J. (1989). Light and electron microscopic detection of (3Galß1,4GlcNAcß1) sequences in asparagine-linked oligosaccharide with the *Datura stramonium* lectin. Histochemistry 92, 515-522.

Evans G.S., Chwalinski S., Owen G., Booth C., Singh A. and Potten C.S. (1994). Expression of pokeweed lectin binding in murine intestinal paneth cells. Epith. Cell Biol. 3, 7-15.

Feizi T. (1981). The blood group Ii system: a carbohydrate antigen system defined by naturally monoclonal or oligoclonal autoantibodies of man. Immunol. Commun. 10, 127-156.

Feizi T. (1992). Blood group-related oligosaccharides are ligands in cell-adhesion events. Biochem. Soc. Transact. 20, 274-278.

Feizi T. and Childs R.A. (1987). Carbohydrates as antigenic determinants of glycoproteins. Biochem. J. 245, 1-11.

Feizi T., Childs R.A., Watanabe K. and Hakomori S. (1979). The types of blood group I specificity among monoclonal anti-I autoantibodies revealed by analogues of a branched erythrocyte glycolipid. J. Exp. Med. 149, 975-980.

Friedman M.J., Fukuda M. and Laine R.A. (1985). Evidence for a malarial parasite interaction site on the major transmembrane protein of the human erythrocyte. Science 228, 75-77.

Fukuda M. (1985). Cell surface glycoconjugates as oncodifferentiation markers in hematopoietic cells. Biochim. Biophys. Acta 780, 119-150.

Fukuda M. (1994). Cell surface carbohydrate: cell-type specific expression. In: Molecular glycobiology. Fukuda M. and Hindsgaul O.

(eds). IRL Press. Oxford pp 1-43.

Fukuda M.N. and Matsumura G. (1976). Endo-ß-galctosidase of *Escherichia freundii*. Purification and endoglycosidic action on keratan sulfates, oligosaccharides, and blood group active glycoprotein. J. Biol. Chem. 251, 6218-6225.

Fukuda M.N., Watanabe K. and Hakomori S. (1978). Release of oligosaccharides from various glycosphingolipids by endo-ß-galactosidase. J. Biol. Chem. 253, 6814-6819.

Fukuda M.N., Fukuda M. and Hakomori S. (1979). Cell surface modification by endo-ß-galactosidase. Change of blood group anctivities and release of oligosaccharides from glycoproteins and glycosphingolipids of human erythrocytes. J. Biol. Chem. 254, 5458-5465.

Fukuda M., Dell A., Oates J.E. and Fukuda M.N. (1984a). Structure of branched lactosaminoglycan, the carbohydrate moiety of band 3 isolated from adult human erythrocytes. J. Biol. Chem. 259, 8260-8273.

Fukuda M., Spooncer E.,, Oates J.E., Dell A. and Klock J.C. (1984b). Structure of sialylated fucosyl lactosaminoglycan isolated from human granulocytes. J. Biol. Chem. 259, 10925-10935.

Fukuda M., Carlsson S.R., Klock J.C. and Dell A. (1986). Structures of O-linked oligosaccharides isolated from normal granulocytes, chronic myelogenous leukemia cells, and acute myelogenous leukemia cells. J. Biol. Chem. 261, 12796-12806.

Gallagher J.T., Morris A. and Dexter T.M. (1985). Identification of two binding sites for wheat-germ agglutinin on polylactosamine-type oligosaccharides. Biochem. J. 231, 115-122.

Gooi H.C., Veyrieres A., Alais J., Scudder P., Hounsell E.F. and Feizi T. (1984). Further studies of the specificities of monoclonal anti-i and anti-I antibodies using chemically synthesized, linear oligosaccharides of the poly-N-acetyllactosamine series. Mol. Immunol. 21, 1099-1104.

Goldstein I.J. and Poretz R.D. (1986). Isolation, physicochemical characterization, and carbohydrate-binding specificity of lectins. In: The lectins properties, functions and applications in biology and medicine. Liener I. Sharon N. and Goldstein I.J. (eds). Academic Press. New York. pp 33-247.

Griffin N.R. and Wells M. (1993). Semiquantitative immunohistochemical studies of blood group antigen A,B, H,. Le^a, Le^b structures and Ii backbone chains in the normal human cervix and in cervical adenocarcinoma. Histochem. J. 25, 228-241.

Hakomori S. (1981). Blood group ABH and Ii antigens of human erythrocytes: chemistry, polymorphism, and their developmental change. Semin. Hematol. 18, 39-62.

Hanisch F-G., Uhlenbruck G., Peter-Katalinic J., Egge H., Dabrowski J. and Dabrowski U. (1989). Structures of neutral O-linked polylactosaminoglycans on human skin milk mucins. J. Biol. Chem. 264, 872-883.

Hanisch F-G., Heimbuchel G., Baldus S.E., Uhlenbruck G., Schmits R., Pfreundschuh M., Schwonzen M., Vierbuchen M., Bara J. and Peter-Katalinic J. (1993). Monoclonal antibody FW6 defines an epitope on α3/4-monofucosylated polylactosaminoglycans expressed by fetal and colon carcinoma-associated mucins. Cancer Res. 53, 4367-4375.

Heffernan M., Lotan R., Amos B., Palcic M., Takano R. and Dennis J.W. (1993). Branching ß1-6N-acetylglucosaminetransferases and polylactosamine expression in mouse F9 teratocarcinoma cells and differentiated counterparts. J. Biol. Chem. 268, 1242-1251.

Hirohashi S., Ino Y., Kodama T. and Shimosato Y. (1984). Distribution of blood group antigens A, B, H, and I (Ma) in mucous-producing adenocarcinoma of human lung. J. Natl. Canc. Inst. 72, 1299-1305.

Hubbard S.C. (1987). Differential effects of oncogenic transformation on N-linked oligosaccharide processing at individual glycosylation sites of viral glycoproteins. J. Biol. Chem. 262, 16403-16411.

Hughes C.M. and Rudland P.S. (1990a). Immunocytochemical identification of myoepithelial cells in normal and neoplastic rat mammary glands with the lectins *Griffonia simplicifolia*-I and pokeweed mitogen. J. Histochem. Cytochem. 38, 1633-1645.

Hughes C.M. and Rudland P.S. (1990b). Appearance of myoepithelial cells in developing rat mammary glands identified with the lectins *Griffonia simplicifolia*-I and pokeweed mitogen. J. Histochem. Cytochem. 38, 1647-1657.

Irimura T. and Nicolson G.L. (1983). Interaction of pokeweed mitogen with poly(N-acetyllactosamine)-type carbohydrate chains. Carbohydr. Res. 120, 187-195.

Irimura T., North S.M. and Nicolson G.L. (1987). Glycoprotein profiles of macrophages at different stages of activation as revealed by lectin binding after electrophoretic separation. Eur. J. Immunol. 17, 73-78.

Itai S., Nishikata J., Takahashi N., Tanaka O., Matsubara Y., Hasegawa S., Yanai N., Takaoka K., Arii S., Tobe T. and Kannagi R. (1990). Differentiation-dependent expression of I and sialyl I antigens in the developing lung of human embryos and in lung cancers. Cancer Res. 50, 7603-7611.

Ito N. and Hirota T. (1992). Histochemical and cytochemical localization of blood group antigens. Progr. Histochem. Cytochem. 25(No. 2), 1-85.

Ito N., Tabata S., Kawahara S., Hirano Y., Nakajima K., Uchida K. and Hirota T. (1993). Histochemical analysis of blood group antigens in human sublingual glands and pancreas. An application of high-performance liquid chromatography to estimate the quantity of galactose liberated from tissue sections by α-galactosidase digestion. Histochem. J. 25, 242-249.

Ito N., Kawahara S., Hirano Y., Morimura Y., Nakajima K., Uchida K. and Hirota T. (1994a). Localization of blood-group-related linear poly-N-acetyllactosamine structure in different human tissues by *Griffonia simplicifolia* agglutinin-II staining following endo-ß-galactosidase digestion. Histochem. J. 26, 327-336.

Ito N., Yokota M., Kawahara S., Morimura Y., Hirano Y., Nagaike C., Uchida K., Hirota T. and Matsunaga T. (1994b). Histochemical demonstration of endo-ß-galactosidase susceptible poly-N-acetyl-lactosamine with the blood group specificities in papillary carcinomas of the human thyroid glands. Acta Histochem. Cytochem. 27, 451-458.

Ito N., Yokota M., Kawahara S., Nagaike C., Morimura Y., Hirota T. and Matsunaga T. (1995). Histochemical demonstration of different types of poly-N-acetyllactosamine structures in human thyroid neoplasms by using lectins and endo-ß-galactosidase digestion. Histochem. J. 27, 620-629.

Ivatt R.J., Reeder J.W. and Clark G.F. (1986). Structural and conformational features that affect the interaction of poly-lactosaminoglycans with immobilized wheat germ agglutinin. Biochim.Biophys. Acta 883, 253-264.

Kannagi R., Nudelman E., Levery S.B. and Hakomori S. (1982). A series of human erythrocyte glycosphingolipids reacting to the monoclonal antibody directed to a developmentally regulated antigen, SSEA-1. J. Biol. Chem. 257, 14865-14874.

Kapadia A., Feizi T. and Evans J.M. (1981a). Changes in the expression and polarization of blood group I and i antigens in post-implantation

embryos and teratocarcinomas of mouse associated with cell differentiation. Exp. Cell Res. 131, 185-195.

Kapadia A., Feizi T., Jewell D., Keeling J. and Slavin G. (1981b). Immunocytochemical studies of blood group A, H, I, and i antigens in gastric mucosae of infants with normal gastric histology and of patients with gastric carcinoma and chronic benign peptic ulceration. J. Clin. Pathol. 34, 320-337.

Kinloch R.A., Mortillo S., Stewart C.L. and Wassarman P.M. (1991). Embryonal carcinoma cells transfected with ZP3 genes differentially glycosylate similar polypeptides and secrete active mouse sperm receptor. J. Cell Biol. 115, 655-664.

Laferte S. and Dennis J.W. (1989). Purification of two glycoproteins expressing ß1-6 branched Asn-linked oligosaccharides from metastatic tumour cells. Biochem. J. 259, 569-576.

Laine R.A. and Rush J.S. (1988). Chemistry of human erythrocyte polylactosamine glycopeptides (erythroglycans) as related to ABH blood group antigenic determinants. Adv. Exp. Med. Biol. 228, 331-347.

Lee N., Wang W-C. and Fukuda M. (1990). Granulocytic differentiation of HL-60 cells is associated with increase of poly-N-acetyllactosamine in Asn-linked oligosaccharides attached to human lysosomal membrane glycoproteins. J. Biol. Chem. 265, 20476-20487.

Lenhard V., Seeling H.P., Geisen H.P. and Roelcke D. (1978). Identification of I/i, Pr_{1-3} and Gd antigens in the human kidney: possible relevance to hyperacute graft rejection induced by cold agglutinins. Clin. Exp. Immunol. 33, 276-282.

Liukkonen J., Haataja S., Tikkanen K., Kelm S. and Finne J. (1992). Identification of N-acetylneuraminyl α 2-3 poly-N-acetyllactosamine glycans as the receptors of sialic acid-binding *Streptococcus suis* strains. J. Biol. Chem. 267, 21105-21111.

Ljungberg O. (1992). Biopsy pathology of the thyroid and parathyroid. Chapman & Hall. London.

Loomes L.M., Uemura K., Childs R.A., Paulson J.C., Rogers G.N., Scudder P.R., Michalski J-C., Hounsell E.F., Taylor-Robinson D. and Feizi T. (1984). Erythrocyte receptors for *Mycoplasma pneumoniae* are sialylated oligosaccharides of Ii antigen type. Nature 307, 560-563.

Loveless R.W. and Feizi T. (1989). Sialo-oligosaccharide receptors for *Mycoplasma pneumoniae* and related oligosaccharides of poly-N-acetyllactosamine series are polarized at the cilia and apical-microvillar domains of the ciliated cells in human bronchial epithelium. Infect. Immunol. 57, 1285-1289.

Loveless R.W., Griffiths S., Fryer P.R., Blauth C. and Feizi T. (1992). Immunoelectron microscopic studies reveal differences in distribution of sialo-oligossaccharide receptors for *Mycoplasma pneumoniae* on the epithelium of human and hamster bronchi. Infect. Immunol. 60, 4015-4023.

Lloyd K.O. (1987). Blood group antigens as markers for normal differentiation and malignant changes in human tissues. Am. J. Clin. Pathol. 87, 129-139.

Maley F., Trimble R.B., Tarentino A.L. and Plummer Jr. T.H. (1989). Characterization of glycoproteins and their associated oligosaccharides through the use of endoglycosidases. Anal. Biochem. 180, 195-204.

Maemura K. and Fukuda M. (1992). Poly-N-acetyllactosaminyl O-glycans attached to leukosialin. J. Biol. Chem. 267, 24379-24386.

Merkle R.K. and Cummings R.D. (1987). Relationship of the terminal sequences of the length of poly-N-acetyllactosamine chains in asparagine-linked oligosaccharides from the mouse lymphoma cell line BW25147. Immobilized tomato lectin interacts with high affinity with glycopeptides containing long poly-N-acetyllactosamine chains. J. Biol. Chem. 262, 8179-8189.

Miyake M., Kohno N., Nudelman E.D. and Hakomori S. (1989). Human IgG_3 monoclonal antibody directed to an unbranched repeating type 2 chain (Galß1-4GlcNAcß1-3Galß1-4GlcNAcß1-3 Galß1-R) which is highly expressed in colonic and hepatocellular carcinoma. Cancer Res. 49, 5689-5695.

Monsigny M., Roche A-C., Sene C., Maget-Dana R. and Delmotte F. (1980). Sugar-lectin interactions: How dose wheat-germ agglutinin bind sialoglycoconjugates. Eur. J. Biochem. 104, 147-153.

Moore K.L., Eaton S.F., Lyons D.E., Lichenstein H.S., Cummings R.D. and McEver R.P. (1994). The P-selectin glycoprotein ligand from human neutrophils displays sialylated, fucosylated, O-linked poly-N-acetyllactosamine. J. Biol. Chem. 269, 23318-23327.

Muramatsu T. (1988). Developmentary regulated expression of cell surface carbohydrates during mouse embryogenesis. J. Cell Biochem. 36, 1-14.

Muramatsu H. and Muramatsu T. (1990). Analysis of glycoprotein-bound carbohydrates from pluripotent embryonal carcinoma cells by pokeweed agglutinin-agarose. J. Biochem. 107, 629-634.

Nudelman E.D., Levery S.B., Stroud M.R., Ellen M., Salyan M.E.K., Abe K. and Hakomori S. (1988). A novel tumor-associated, developmentally regulated glycolipid antigen defined by monoclonal antibody ACFH-18. J. Biol. Chem. 263, 13942-13951.

Pennington J.E., Rastan S., Roelcke D. and Feizi T. (1985). Saccharide structures of the mouse embryo during the first eight days of development. Inferences from immunocytochemical studies using monoclonal antibodies in conjunction with glycosidases. J. Embyrol. Exp. Morphol. 90, 335-361.

Picard J., Edward D.W. and Feizi T. (1978). Changes in the expression of the blood group A, B, H, Le^a and Le^b antigens and the blood group precursor associated I(MA) antigen in glycoprotein-rich extracts of gastric carcinomas. J. Clin. Lab. Immunol. 1, 119-128.

Rabinowitz S.S. and Gordon S. (1991). Macrosialin, a macrophage-restricted membrane sialoprotein differentially glycosylated in response to inflammatory stimuli. J. Exp. Med. 174, 827-836.

Raghunath M., Grupp C., Newmann I., Heidtmann A. and Roelcke D. (1994). Polylactosamine sugar chains expressed by epithelia of Henle's loop and collecting duct in rat and human kidney are selectively recognized by human cold agglutinins anti-I/i. Tissue Antigen 44, 159-165.

Römer W., Seelig H.P., Lenhard V. and Roelcke D. (1979). The distribution of I/i, Pr and Gd antigens in mammalian tissues. Invest. Cell Pathol. 2, 157-162.

Saitoh O., Wang W-C., Lotan R. and Fukuda M. (1992). Differential glycosylation and cell surface expression of lysosomal membrane glycoproteins in sublines of a human colon cancer exhibiting distinct metastatic potentials. J. Biol. Chem. 267, 5700-5711.

Schwonzen M., Schmits R., Baldus S.E., Vierbuchen M., Hanisch F-G., Pfreundschuh M., Diehl V., Bara J. and Uhlenbruck G. (1992). Monoclonal antibody FW6 generated against a mucin-carbohydrate of human amniotic fluid recognizes a colonic tumor-associated epitope. Br. J. Cancer 65, 559-565.

Scudder P., Hanfland P., Uemura K. and Feizi T. (1984). Endo-ß-D-galactosidases of *Bacteroides fragilis* and *Escherichia freundii* hydrolyze linear but not branched oligosaccharide domains of glycolipids of the neolactto series. J. Biol. Chem. 259, 6586-6592.

Spillmann D. and Finne J. (1994). Identification of a major poly-N-acetyllactosamine-containing cell-surface glycoprotein of mouse teratocarcinoma cells. Appearance of cells induced to primitive endoderm but not parietal endoderm differentiation. Eur. J. Biochem. 220, 385-394.

Szulman A.E. (1964). The histological distribution of the blood group substances in man as disclosed by immunofluorescence. III. The A, B, and H antigens in embryos and fetuses from 18 mm in length. J. Exp. Med. 119, 503-516.

Tsay G.C., Dawson G. and Li Y.-T. (1975). Structure of the glycopeptide storage material in GM1 gangliosidosis. Sequence determination with specific endo- and exoglycosidases. Biochem. Biophys. Acta 385, 305-311.

Vierbuchen M., Schröder S., Uhlenbruck G., Ortmann M. and Fischer R. (1989). CA 50 and CA 19-9 antigen expression in normal, hyperplastic, and neoplastic thyroid tissue. Lab. Invest. 60, 726-732.

Vierbuchen M., Larena A., Schröder S., Hanisch F-G., Ortmann M., Larena A., Uhlenbruck G. and Fischer R. (1992). Blood group antigen expression in medullary carcinoma of the thyroid. Virchows Arch. (B) 62, 79-88.

Vowden P., Lowe A.D., Lennox E.S. and Bleehen N.M. (1986). Thyroid blood group isoantigen expression: a parallel with ABH isoantigen expression in the distal colon. Br. J. Cancer 53, 721-725.

Wang W-C., Lee N., Aoki D., Fukuda M.N. and Fukuda M. (1991). The poly-N-acetyllactosamines attached to lysosomal membrane glycoproteins are increased by the prolonged association with the Golgi complex. J. Biol. Chem. 266, 23185-23190.

Yamashita K., Ohkura T., Tachibana Y., Takasaki S. and Kobata A. (1984). Comparative study of the oligosaccharides released from baby hamster kidney cells and their polyoma transformant by hydrazinolysis. J. Biol. Chem. 259, 10834-10840.

Yamashita K., Totani K., Ohkura T., Takasaki S., Goldstein I.J. and Kobata A. (1987). Carbohydrate binding properties of complex-type oligosaccharides on immobilized *Datura stramonium* lectin. J. Biol. Chem. 262, 1602-1607.

Yokota M., Ito N., Hirota T., Yane K., Tanaka O, Miyahara H. and Matsunaga T. (1995). Histochemical differences of the lectin affinities of backbone polylactosamine structures carrying the ABO blood group antigens in papillary carcinoma and other types of thyroid neoplasm. Histochem. J. 27, 139-147.

Zhu B.C.-R. and Laine R.A. (1989). Purification of acetyllactosamine-specific tomato lectin by erythroglycan-sepharose affinity chomatography. Prep. Biochem. 19, 341-350.