Differential expression of β1,3galactosyltransferases in human colon cells derived from adenocarcinomas or normal mucosa[1]

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Abstract Two β1,3galactosyltransferases are detected in human colon cells: one corresponds to β3GalT1, the other (β3GalTx) is found to be different from any cloned β3GalT since in vitro it utilizes GlcNAc very efficiently under specific reaction conditions. Expression of β3GalT1 transcript is high in normal colon mucosa and control neuroectodermal cells, which do not express sialyl-Lewis a antigen, and low in colon adenocarcinoma cells, as assessed by competitive RT-PCR. β3GalTx activity is high in adenocarcinoma cells expressing sialyl-Lewis a and undetectable in all other cells, suggesting differential involvement and opposite regulation of such enzymes during carcinogenesis.

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Key words: Galactosyltransferase; Type 1 chain; Sialyl-Lewis a antigen; Carcinoembryonic antigen; Colon adenocarcinoma; Competitive PCR

1. Introduction

There is increasing evidence that glycosyltransferases are coded by families of multiple related genes whose expression is carefully regulated in different tissues and in different physiological or pathological states [1–4]. The Galβ1-3GlcNAc disaccharide is the core sequence (type 1 structure) of oncodevelopmental antigens such as sialyl-Le^a or Le^b [5,6], but is also reported in the saccharide chain of normal tissue glycoproteins [7,8], and as a target of auto antibodies in neuropathies [9,10]. The enzymes involved in its biosynthesis are still not well defined and their regulation is totally unknown. Four members of the β1,3GalT family have been recently cloned [11]. β3GalT1, β3GalT2, and β3GalT3 are considered to be able to synthesize the type 1 structure, while it is though that β3GalT4 is able to synthesize the Galβ1-3GalNAc sequence. β3GalT1 and β3GalT2 use GlcNAc as acceptor in vitro very poorly, and β3GalT3 does not use it at all. Conversely, a β1,3GalT activity expressed in human colon adenocarcinoma cells was found to efficiently use GlcNAc as acceptor, but only at very high concentrations, and to require rather high concentrations of donor UDP-Gal too [12].

In this work, we investigated whether any cloned β3GalT corresponds to the enzyme we detected in human colon adenocarcinoma cells, and whether the expression is related to that of type 1 chain Lewis antigens. To this end, we determined the expression levels of cloned β3GalTs by Northern blot and RT-PCR in human colon adenocarcinoma cell line COLO-205, that expresses Lewis type 1 antigens and β1,3GalT activity [12]. On the basis of the results, we used competitive RT-PCR to quantitate the RNA transcripts of β3GalT1 and two glycosyltransferases involved in the synthesis of type 1 chain Lewis antigens (Fuc-TIII and FUT2), in COLO-205 and other human cells derived from colon adenocarcinomas and normal mucosa. We also screened a COLO-205 cDNA library by colony hybridization using a β3GalT1 fragment as a probe, and studied the GalT activity directed by an isolated cDNA clone, upon expression in CHO cells, by immunostaining and in vitro enzyme assay.

2. Materials and methods

2.1. Materials

GlcNAc, GlcNAcβ1-O-benzyl (benzyl-GlcNAc), GlcNAcβ1-O-phenyl (phenyl-GlcNAc), GlcNAcβ1-3Galβ1-O-methyl, GlcNAcβ1-6Manα1-O-methyl, GlcNAcβ1-6GlcNAc, UDP-Gal, α-lactalbumin, egg ovalbumin, bovine asialomucin, asialofetuin, and FITC conjugate goat anti-mouse IgG were from Sigma. UDP-[6-^{3}H]Gal was from Amersham. Anti-sialyl-Le^a (0.9 mg/ml) monoclonal antibody was prepared by protein A-Sepharose affinity chromatography of the culture media of hybridoma 1116-NS-19-9 (ATCC HB-8059).

2.2. Cell lines

Human colon adenocarcinoma COLO-205, HCT-15 and HT-29 cells were cultured as previously reported [12]. Human colon normal mucosa cells FHC (ATCC CRL-1831) were cultured in Dulbecco's modified Eagle's medium/Ham F:12 (1:1) mixture containing 10% fetal bovine serum, 100 U/ml penicillin, 100 mg/ml streptomycin, 2 mM L-Glu, and supplemented with 10 ng/ml cholera toxin, 5 μg/ml insulin, 5 μg/ml transferrin, and 100 ng/ml hydrocortisone. Human neuroblastoma IMR-32 (ATCC CCL-127) and melanoma WM266-4 (ATCC CRL-1676) cells were cultured in Eagle's minimum essential medium containing 10% fetal bovine serum, 100 U/ml penicillin, 100 mg/ml streptomycin, 2 mM L-Glu, and supplemented with 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate, and Earle's balanced salt solution. CHO cells expressing Polyoma virus T antigen and human Fuc-TIII (CHO-T-FT), a gift of J.B. Lowe (University of Michigan), were cultured in minimum essential medium (α-modification) containing 10% fetal bovine serum, 100 U/ml penicillin, 100 mg/ml streptomycin, 2 mM L-Glu, and supplemented with 0.5 mg/ml (active) G418 and 750 U/ml Hygromycin B. They were transiently transfected with plasmid DNA using the procedure reported [13].

2.3. Isolation of a cDNA clone

Total and poly(A)$^+$ RNAs were prepared as reported [12]. For COLO-205 cDNA library construction, poly(A)$^+$ RNA was reverse

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[1] The complete sequence of the cDNA reported in this paper (COLO-205 β3GalT1) has been submitted to GenBank with accession number AF117222.

Abbreviations: Le^a, Lewis a, Galβ1-3[Fucα1-4]GlcNAc; sialyl-Le^a, sialyl-Lewis a, NeuAcα2-3Galβ1-3[Fucα1-4]GlcNAc; Le^b, Lewis b, Fucα1-2Galβ1-3[Fucα1-4]GlcNAc; CEA, carcinoembryonic antigen; NCA-2, non-specific cross-reacting antigen 2; NFA-2, normal fecal antigen 2; FUT2, secretor type α1,2fucosyltransferase; Fuc-TIII, α1,3/1,4fucosyltransferase; GalT, galactosyltransferase; RT-PCR, reverse transcriptase mediated-PCR

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transcribed in separate reactions as follows. Reaction 1 contained, in 40 μl volume, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 5 mM $MgCl_2$, 1000 U/ml human placental ribonuclease inhibitor, 1 mM of each deoxynucleotide triphosphate, 2.5 μM random hexanucleotide primers, 2500 U/ml Moloney Murine Leukemia virus (MuLV) reverse transcriptase (Perkin Elmer), and 50 mg/ml freshly denatured (5 min at 65°C, 2 min on ice) poly(A)$^+$ RNA.

Reaction 2 contained, in 40 μl volume, 50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 1000 U/ml human placental ribonuclease inhibitor, 1 mM of each deoxynucleotide triphosphate, 3.5 μM random hexanucleotide primers, 2500 U/ml MuLV reverse transcriptase (Amersham), and 50 mg/ml freshly denatured poly(A)$^+$ RNA.

Reaction 3 contained, in 40 μl volume, 50 mM Tris-HCl pH 8.3, 50 mM KCl, 5 mM $MgCl_2$, 1000 U/ml human placental ribonuclease inhibitor, 1 mM of each deoxynucleotide triphosphate, 3.5 μM random hexanucleotide primers, 1000 U/ml Avian Myeloblastosis virus reverse transcriptase (Amersham), and 50 mg/ml freshly denatured poly(A)$^+$ RNA. Reactions were kept 10 min at room temperature, 40 min at 37°C and 45 min at 42°C. Second strand cDNA was synthesized from the obtained first strand cDNA (0.1 ml equal mixture of each reaction) by a commercial kit (cDNA plus, Amersham) following the manufacturer's recommendations. Blunt ended cDNA was ligated to a *Bst*XI adapter (upper strand: 5′-GCTTCTCGAGCTTTAGAG-CACA; lower strand: 5′-CTCTAAAGCTCGAGAAGC), size fractionated by a double passage on a Sephacryl 500 column (Gibco), cloned in the *Bst*XI sites of pCDM8 vector, and the ligated material electroporated into *E. coli* strain MC1061/P3. Calculated library size was greater than 10^7 recombinants per 1 μg starting RNA, and background lower than 1%. The average insert size, determined by *Xho*I digestion of plasmid DNA extracted from library pools of 500, 2000, and 5000 recombinants, was 1.5 Kbp. Primary recombinants obtained upon electroporation were plated on agar plates (about 10 000 colonies per 150 mm plates) and screened by conventional colony hybridization using a [^{32}P]-labeled β3GalT1 probe. Probe was the 743 bp PCR product reported in Table 1. Blots were washed in 2×SSC (0.30 M NaCl and 0.03 M sodium citrate) containing 0.5% SDS for 15 min at room temperature (twice), and then for 30 min at 55°C. A 1.9 Kbp insert isolated from a single positive colony (COLO-205 β3GalT1) was sequenced by the dideoxy chain termination method using an automated procedure.

2.4. RT-PCR and competitive RT-PCR

First strand cDNA was prepared from poly(A)$^+$ RNA as for cDNA library construction (reaction 2), and an amount corresponding to 0.25 μg of reverse transcribed RNA (5 μl of cDNA synthesis reaction) submitted to PCR amplification. Standard amplification reaction contained, in a total volume of 50 μl, 0.1 mM of each deoxynucleotide triphosphate, 10 mM Tris/HCl, pH 8.8, 50 mM KCl, 1 mM $MgCl_2$, 1% Triton X-100, 250 ng of each primer (see Table 1), and 2.5 U of Taq polymerase (Eurobio). Reactions were incubated as follows: a single treatment at 94°C for 2.0 min, followed by a cycle consisting of 1.5 min at 94°C (melting) and 3.5 min at 72°C (annealing plus extension), that was repeated 25 or 35 times. A final extension step was performed at 72°C for 8 min. Oligonucleotide primer pairs were designed on the basis of published sequences as reported in Table 1. For competitive RT-PCR [14], first strand cDNA was prepared under the same conditions as reported above (reaction 2), but using 0.4 μM oligo(dT)$_{16}$ as primer and freshly denatured total RNA (250 μg/ml) as template. In preliminary experiments, 1 μl aliquots of the cDNA synthesis reactions were mixed with different amounts of linear competitors (from 1 fg to 100 pg) and submitted to standard PCR amplification for 25–35 cycles. On the basis of the obtained results, different amounts of cDNA (0.5–2.0 μl of first strand synthesis reactions) were then mixed with 45 pg of β-actin linear competitor cDNA and submitted to PCR amplifications for 25 cycles, until the amount of cDNA that provides comparable results was selected (Fig. 2, upper part). In the final experiments, normalized cDNA aliquots were mixed with single amounts of each competitor (25 fg for β3GalT1, 100 fg for Fuc-TIII, and 50 fg for FUT2), and submitted to PCR amplification (35 cycles for β3GalT1, 30 cycles for Fuc-TIII and FUT2). In all cases, parallel PCR amplifications were performed on known amounts of standard linear cDNAs pre-mixed with the competitors. Aliquots (10 μl) of PCR reactions were analyzed by 1% agarose gel electrophoresis and visualized by staining with ethidium bromide under UV light. Competitor cDNAs were prepared by removing short restriction fragments from the regions of amplification as follows: a 280 bp fragment was removed from β-actin by *Rca*I digestion, a 279 bp fragment from β3GalT1 by *Eco*RV/*Xmn*I digestion, a 136 bp fragment from Fuc-TIII by *Kpn*I digestion, and a 196 bp fragment from FUT2 by *Bam*HI/*Pst*I digestion (Table 1). Truncated cDNAs were self-ligated, cloned, linearized, quantitated, and diluted.

Northern blotting was performed on denatured poly(A)$^+$ RNA (3 μg) as reported [12].

2.5. Enzyme assays

Cells were harvested, washed, and resuspended as described [12]. β3GalT1 was determined in a reaction mixture containing, in a final volume of 20 μl, 0.1 M HEPES buffer, pH 7.4, 10 mM $MnCl_2$, 0.5 mg/ml Triton X-100, 1 mg/ml α-lactalbumin, 60 μM donor UDP-[^{3}H]Gal, specific radioactivity 20 mCi/mmol, 40 mM acceptor GlcNAc, and 0.2–2.0 mg/ml cell protein. Incubation was done at 37°C for 90 min. β3GalTx was determined in a reaction mixture as β3GalT1 but containing 1.0 mM donor UDP-[^{3}H]Gal, specific radioactivity 5 mCi/mmol, 0.8 M acceptor GlcNAc, and 0.1–2.0 mg/ml cell protein. Incubation was done at 37°C for 60 min. Reaction products were assayed by Dowex chromatography (saccharide acceptors) or phosphotungstic acid precipitation (glycoprotein acceptor), and radioactivity incorporation was determined by liquid scintillation counting as reported [12].

2.6. Immunofluorescence and panning

Cells were detached, recovered, washed, and stained in suspension as reported [12], using 1:500 dilution of monoclonal antibodies anti-sialyl-Le^a or anti-Le^a. Alternatively, cells grown on tissue culture slides were washed with PBS, fixed with 3.7% formaldehyde in PBS, washed three times with PBS, and stained as above for cell suspensions. Fluorescence was determined by flow cytometry and microscopy.

For panning, CHO-T-FT cells transfected with β3GalT1 cDNA were detached from plates (100 mm), washed, suspended in staining medium (4 ml/transfected plate), and placed on panning dishes (4 ml/dish), prepared by covering Petri dishes with anti-sialyl-Le^a antibody, as reported [13]. Panning was performed at 4°C for 1 h. Non-adherent cells were removed by washing three times with PBS, while adherent cells were detached, washed, and processed.

3. Results

3.1. Detection of β1,3GalT transcripts in human colon adenocarcinoma cells COLO-205

To determine whether the β1,3GalT activity detected in COLO-205 cells is due to one of the cloned β3GalT genes, COLO-205 cDNA was amplified by PCR using oligonucleotide primers deduced from the reported sequences (Table 1). Amplification was detected with β3GalT1 primers, while only

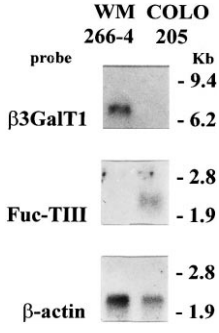

Fig. 1. Northern blot analysis of COLO-205 and control WM266-4 cells. Poly(A)$^+$ RNA was separated by formaldehyde gel electrophoresis, and blotted onto a nitrocellulose membrane that was probed with [^{32}P]-labeled β3GalT1 probe. The same membrane was sequentially stripped and reprobed using [^{32}P]-labeled Fuc-TIII and β-actin probes, respectively.

Table 1
Oligonucleotide primers used for RT-PCR analyses

RNA	Primers	Size (bp) of amplified DNA		GenBank accession
		Target	Competitor	
β-actin	U 5′-CTGAACCCCAAGGCCAACCGCGAG	754	474	AB004047
	L 5′-CCTGCTTGCTGATCCACATCTGCTGGAAG			
β3GalT1	U 5′-GCTGGGCCAGCGCTCTCTGGTAC	743	464	E07739
	L 5′-GTGAAGCAGCCTTGTGTGGAGTGAG			
β3GalT2	U 5′-GCCAAAAGGTCTCTGTTCCGCACTC	817		Y15014
	L 5′-GATTGGGTGCATATCCTCGCATTAGG			
β3GalT3	U 5′-ATGGCCTCGGCTCTCTGGACTGTCC	768		Y15062
	L 5′-CATTTCATAGATCCTTGGCACCAAATCTCTGGAC			
Fuc-TIII	U 5′-GATCTGGTTCAACTTGGAGCCACCCCCT	750	614	X53578
	L 5′-CAGCAGGTGAGGCCCCAGGCAG			
FUT2	U 5′-GCGGCTAGCGAAGATTCAAGCCATG	718	522	U17894
	L 5′-GCCCTCAATGCCATCGCCAGCAAACAC			

U, upper strand primer; L, lower strand primer.

a faint spot was obtained with β3GalT2 primers, and no signal with β3GalT3 primers (not shown). COLO-205 poly(A)$^+$ RNA was analyzed by Northern blot using a β3GalT1 probe, but no spot was detected in this case, suggesting low expression of β3GalT1 (Fig. 1). A random hexamer primed cDNA library prepared from COLO-205 cells was screened at low stringency by colony hybridization using the same β3GalT1 probe. A positive colony was isolated and the obtained 1.9 Kbp cDNA insert subcloned in pcDNAI vector. Direct DNA sequencing (GenBank accession AF117222) indicated the presence of an open reading frame of 978 bp whose translation predicted an amino acid sequence identical to β3GalT1 (GenBank accession E07739), confirming the expression of the enzyme in COLO-205.

3.2. β1,3GalT activities expressed in colon cells

Cloned β3GalT1 was expressed in CHO-T-FT cells, a line able to replicate plasmids with the Polyoma origin of replication and synthesize α1,4fucosyl linkage through expression of Polyoma virus large T antigen and human Fuc-TIII, respectively. Transiently transfected cells react with anti-sialyl-Le^a and Le^a antibodies as assessed by immunofluorescence, while mock transfected cells do not. β3GalT1 transfected cells expressing sialyl-Le^a antigen were isolated by panning, and used as enzyme source for determining β1,3GalT activity towards GlcNAc as acceptor. At concentrations of 40 mM GlcNAc and 60 μM UDP-Gal, a weak α-lactalbumin insensitive activity is detected in such cells, but not in mock transfected CHO-T-FT cells, as expected for β3GalT1. The activity is not stimulated but rather inhibited by higher GlcNAc concentrations, while that expressed in COLO-205 cells (β3GalTx) is strongly stimulated. WM266-4 cells, a melanoma cell line known to express high levels of β3GalT1, show similar behaviour to the β3GalT1 transfected CHO-T-FT cells (Table 2). Interestingly, a similar α-lactalbumin insensitive galactosyltransferase activity, not stimulated by high GlcNAc concentration, is present in FHC cells, a line derived from normal human colon mucosa. This suggests the presence of β3GalT1 in this cells too. However, conclusive evidence for the actual β1,3 nature of the activity is lacking since the low amount of product formed did not allow for its chemical characterization. β1,3GalT activity was thus determined in the same cells using high GlcNAc and UDP-Gal concentrations, as previously established in COLO-205 cells. The results indicate that under such conditions genuine β3GalTx activity is detected in COLO-205 and HT-29 cells only, but not in FHC or HCT-15 cells, nor in WM266-4 or IMR-32 cells (Table 2). β3GalTx is found to efficiently use some oligosaccharide acceptors, mostly GlcNAcβ1-3Galβ1-O-methyl, but not others such as benzyl-GlcNAc, or any tested glycoprotein (Table 3). These data indicate that β3GalTx is present in some colon adenocarcinoma cell lines but is not detectable in cells from normal colon mucosa or neuroectodermal origin, and is functionally different from β3GalT1 and β3GalT2.

3.3. Quantitation of β3GalT1 transcript in different cells by competitive RT-PCR

To determine the expression level of β3GalT1 in the different cells studied, total RNA was reversed transcribed using an oligo(dT) primer, and the amount of obtained cDNA normal-

Table 2
Galactosyltransferase specific activity measured in transfected and control cells under different reaction conditions

Cells	Donor UDP-Gal	60 μM			1.0 mM
	acceptor GlcNAc	40 mM		600 mM	800 mM
	α-lactalbumin	−	+	+	+
β3GalT1 transfected sialyl-Le^a positive CHO-T-FT		17.7	8.7	5.7	<0.5
mock transfected sialyl-Le^a negative CHO-T-FT		11.5	<0.5	<0.5	<0.5
COLO-205		48.8	10.9	145	360
HT-29		n.d.	n.d.	n.d.	25
FHC		5.0	2.5	2.1	<0.5
IMR-32		n.d.	n.d.	n.d.	<0.5
WM266-4		4.8	4.1	3.2	<0.5

Values are expressed as nanomoles/mg protein/h transferred Gal.
N.d., not determined.

Table 3
Acceptor specificity of β3GalTx

Acceptor	mM	COLO-205 (0.1 mg/ml cell protein)	HCT-15 (1.0 mg/ml cell protein)
GlcNAc	800	42.9	< 0.5
Benzyl-GlcNAc	40	7.7	0.8
	10	0.9	3.5
Phenyl-GlcNAc	40	22.3	< 0.5
	10	12.7	2.0
GlcNAcβ1-3Galβ1-O-methyl	10	68.7	10.3
	2.5	31.3	4.3
GlcNAcβ1-6Manα1-O-methyl	40	20.6	< 0.5
	10	10.9	1.1
GlcNAcβ1-6GlcNAc	10	11.1	1.4
	mg/ml		
Ovalbumin	10	0.9	3.2
Asialomucin	10	1.5	4.6
Asialofetuin	10	< 0.5	< 0.5

Values are expressed as nanomoles/ml/h transferred Gal. Enzyme activity is considered to be β3GalTx related only when levels in COLO-205 cells are higher than levels obtained using excess of HCT-15 protein.

ized with respect to β-actin as described in Section 2 (Fig. 2 upper part). Normalized cDNA aliquots were then mixed with linear competitor cDNAs corresponding to truncated forms of β3GalT1, Fuc-TIII, and FUT2, respectively, submitted to PCR amplification, and the obtained products analyzed by gel electrophoresis (Fig. 2).

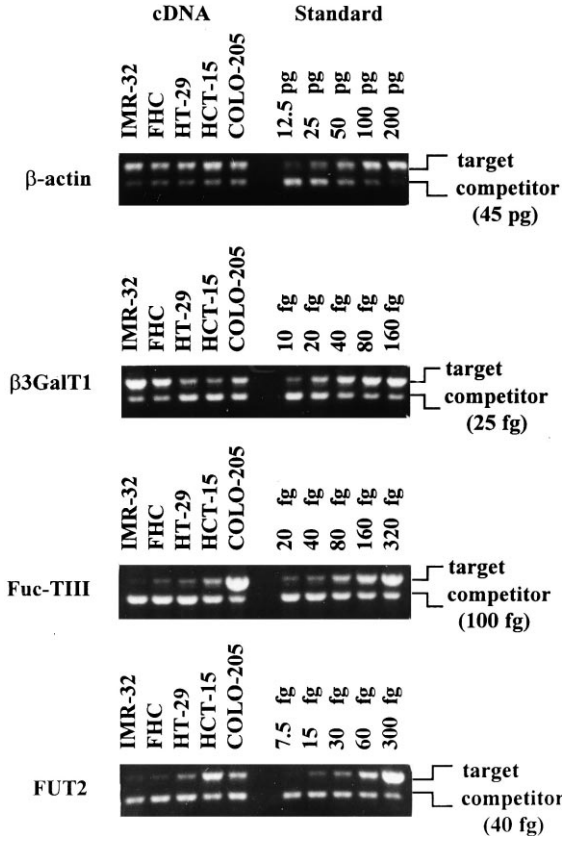

Fig. 2. Competitive RT-PCR analysis of glycosyltransferase transcripts in different cells. Single-stranded cDNA reverse transcribed from total RNA of different cells, or standard linear cDNAs, were mixed with competitor (truncated) cDNAs and subjected to 25 cycles (β-actin), 35 cycles (β3GalT1), or 30 cycles (Fuc-TIII and FUT2) of PCR. Primers and PCR product length are indicated in Table 1. One fifth aliquot of each amplification reaction was electrophoresed in 1% agarose gel and visualized by staining with ethidium bromide.

β3GalT1 transcript is expressed at low or very low levels in the examined human colon adenocarcinoma cell lines, while it is expressed at a much higher level in FHC cells, the line derived from normal human colon mucosa. The expression level in FHC is comparable to that measured in a neuroblastoma cell line. In parallel experiments performed using the corresponding primers and competitors, Fuc-TIII transcript was absent in neuroblastoma cells, but was expressed in HCT-15 and HT-29 adenocarcinoma cells, as well as in FHC cells, and the expression was very high in COLO-205. FUT2 was found to be expressed mostly in HCT-15 and COLO-205, and was also detectable in HT-29, FHC, and even neuroblastoma cells. The quantitative results are summarized in Table 4.

3.4. Expression of sialyl-Le^a and Le^a antigens on the cell surface

To determine the involvement of the different β1,3GalTs in the biosynthesis of Lewis type 1 antigens, their expression was determined by immunofluorescence using mouse anti-sialyl-Le^a and anti-Le^a monoclonal antibodies, and secondary FITC conjugate anti-mouse antibody (Fig. 3). Fluorescence with both anti-sialyl-Le^a and anti-Le^a antibodies is very bright in COLO-205 cells, and moderately bright in HT-29 cells, while is totally negative in FHC, HCT-15, IMR-32, and WM266-4 cells. When these results are compared to β3GalT activity and expression, it appears that the presence of sialyl-Le^a and Le^a antigens on the cell surface is not related to β3GalT1 expression, but rather depends on β3GalTx activity.

Table 4
Quantification of glycosyltransferase transcripts in different cells by competitive RT-PCR

Transcript	Cells				
	IMR-32	FHC	HT-29	HCT-15	COLO-205
β-actin	1000	1000	1000	1000	1000
β3GalT1	1.59	1.42	0.17	0.16	0.41
Fuc-TIII	< 0.05	0.28	0.21	0.72	11.7
FUT2	0.10	0.19	0.19	0.74	0.52

Quantification was performed by densitometric scanning of the negative films of gels in Fig. 2. The amounts of amplified target cDNAs were calculated from their respective standard curves and normalized by those for β-actin.

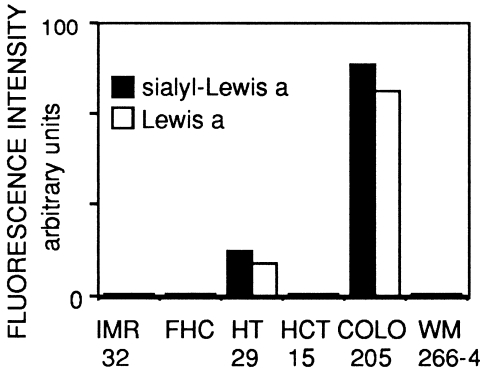

Fig. 3. Immunofluorescence detection of sialyl-Lewis a and Lewis a antigens on the surface of different cells. Antigen expression was determined using anti-sialyl-Lewis a and anti-Lewis a monoclonal antibodies as described Section 2.

4. Discussion

We found that at least two different β1,3GalTs are expressed in human colon mucosa. One is β3GalT1, that is more highly expressed in normal mucosa than in adenocarcinoma cell lines, and the other, that we recently reported [12] as a counterpart of a purified pig trachea β1,3GalT [15], is expressed in some colon adenocarcinoma cells but not in normal mucosa. Such β1,3GalT activity efficiently utilizes GlcNAc as acceptor but only at very high concentrations, and also requires rather high concentrations of UDP-Gal as donor, while β3GalT1 does not efficiently use GlcNAc as acceptor and requires low UDP-Gal concentrations, as previously reported [11,12]. Moreover, the acceptor specificity of this β1,3GalT is different from that reported for β3GalT1 and β3GalT2 [11]. It thus appears to be a distinct enzyme (β3GalTx) and we propose it as a fifth member of the β3GalT gene family. Its specific activity correlates with the amount of Le^a and sialyl-Le^a antigens on the cell surface, while the expression of β3GalT1 transcript does not.

In a previous report, no cloned β3GalT was detected in human colon adenocarcinoma cells by Northern blot [11]. We found that in COLO-205 cells β3GalT1 is well detected by RT-PCR, but not by Northern blot, and that a very faint spot is also formed using β3GalT2 primers, while β3GalT3 is not detected at all. We also isolated a cDNA clone from a COLO-205 cDNA library that was found to be virtually identical to the reported β3GalT1 sequence. Taken together these data indicate that β3GalT1 expression is not restricted to cells of neuroectodermal origin but includes cells of epithelial origin. In particular, while β3GalT1 expression is low in colon adenocarcinoma cells, it is much higher in FHC cells, which are derived from human fetal normal mucosa and considered to be most probably epithelial in nature. Interestingly, FHC cells do not express Le^a or sialyl-Le^a antigens, even if they express a low but detectable amount of Fuc-TIII in addition to β3GalT1, and do not express detectable levels of β3GalTx. Analogously, IMR-32 and WM266-4 cells, that express high levels of β3GalT1 transcript but no detectable β3GalTx activity, lack sialyl-Le^a and Le^a expression. However these cells do not express detectable Fuc-TIII. Moreover, the human colon adenocarcinoma cell line HCT-15, that expresses β3GalT1 and even more Fuc-TIII, but no detectable β3GalTx, does not present any detectable sialyl-Le^a or Le^a antigens on the surface. Taken together these results indicate that β3GalTx is involved in the biosynthesis of type 1 chain Lewis antigens in vivo, while β3GalT1 is not. On the other hand, expression of cloned β3GalT1 in CHO-T-FT cells led to synthesis of sialyl-Le^a and Le^a. Since CHO cells express large amounts of N-glycans containing lactosamine repeats [16], while sialyl-Le^a and Le^a are mostly carried by mucins in human colon adenocarcinomas [17–19], we believe that the differential expression of β3GalT1 and β3GalTx affects distinct glycosylation pathways involving discrete acceptor molecules. In this context, it is worth noting that the normal counterparts of CEA, NFA-2 and NCA-2, contain more Galβ1-3GlcNAc residues than CEA on their sugar chains, and that such residues mostly form the Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-R outer chain sequence [20–22]. This difference was found to be associated with a lower expression of a β1,3GalT activity in normal colon mucosa than in adenocarcinomas, as measured with GlcNAcβ1-3Galβ1-4Glc as acceptor [23]. In conclusion, we suggest that β3GalT1 is down-regulated in colon adenocarcinomas and is a candidate enzyme involved in β1,3galactosylation of complex type N-glycans such as those present on NFA-2, NCA-2, and CEA, while β3GalTx presumably acts on mucin substrates and is up-regulated in colon adenocarcinomas expressing type 1 chain tumor markers. Up-regulation of other glycosyltransferases in human colon cancer has been recently reported using the competitive RT-PCR method [24].

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