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The expression of human FUT1 in HT-29/M3 colon cancer cells instructs the glycosylation of MUC1 and MUC5AC apomucins

Anna López-Ferrer and Carme de Bolós*

Unitat de Biologia Cel-lular i Molecular, Institut Municipal d'Investigació Mèdica, 08003 Barcelona, Spain

Recently, we have reported that in normal gastric epithelium, the expression of gastric apomucins MUC5AC and MUC6 is associated with the specific expression of type 1 and type 2 Lewis antigens, and FUT2 and FUT1 fucosyltransferases, respectively. Until now, there are no data demonstrating the direct implication of specific glycosyltransferases in the specific patterns of apomucin glycosylation.

HT29/M3 colon cancer cell line express MUC1, MUC5AC, type 1 Lewis antigens and FUT2 but not type 2 structures and FUT1, as it occurs in the epithelial cells of the gastric superficial epithelium. These cells were transfected with the cDNA of human FUT1, the α-1,2-fucosyltransferase responsible for the synthesis of type 2 Lewis antigens, to assess the implication of FUT1 in the glycosylation of MUC1 and MUC5AC.

The M3-FUT1 clones obtained express high levels of type 2 Lewis antigens: H type 2 and Ley antigens. Immunoprecipitation of MUC1 and MUC5AC apomucins gives the direct evidence that FUT1 catalyses the addition of α-1,2-fucose to these apomucins, supporting the hypothesis that the pattern of apomucin glycosylation is not only instructed by the mucin primary sequence but also by the set of glycosyltransferases expressed in each specific cell type.

Keywords: **apomucin glycosylation, fucosyltransferases, MUC1, MUC5AC**

Introduction

Mucins are highly O-glycosylated proteins that are the main component of the mucus that covers the surface of all the epithelial tissues. Until now, cDNAs coding for eleven different human mucin genes have been cloned (MUC1-MUC4, MUC5B, MUC5AC, MUC6-MUC8, MUC11-MUC12) [1–5]. Each of these genes displays a characteristic pattern of expression, nevertheless, none of these gene products is restricted to a single tissue or cell type [6,7].

The heterogeneity associated to the broad possibilities of glycosylation of apomucins suggests different functions for mucins in each of the tissues where they are expressed. Also, alterations in the glycosydic component of the mucins may imply the loss of their functionality or the acquisition of new capacities, as is the ability to escape from the immune system [8] and the increased capacity of invasion that acquire certain tumor cells [9]. It has been reported on nuclear magnetic resonance studies [10] that the O-glycans attached to the MUC1 apomucin confer a rigid and long extending structure to the protein core. These properties increase the invasive and metastatic potential of tumor cells [11–13].

Lewis antigens are terminal fucosylated oligosaccharides that are synthesised by the sequential action of specific glycosyltransferases. The family of α-1,2-fucosyltransferases catalyse the addition of fucose in α-1,2-linkage to the galactose of type 1(Galβ1,3GlcNAc-R) and type 2 (Galβ1,4GlcNAc-R) disaccharide to form H type 1 and H type 2 antigens, respectively. Two human α-1,2-fucosyltransferases have been cloned: FUT1 [14] and FUT2 [15] also named H and Secretor genes respectively. α2,3-sialyltransferases catalyse the addition of sialic acid to the same positions in competition with α-1,2-fucosyltransferases. The human α-1,3/1,4-fucosyltransferases that catalyse the addition of fucose to GlcNAc residues in type 1 and type 2 precursors are FUT3 [16], FUT4 [17], FUT5 [18], FUT6 [19] and FUT7 [20]. Among them, FUT3 is the Lewis gene, expressed in epithelial tissues and with α-1,3 and α-1,4-fucosyltransferase activity.

The presence of Lewis antigens in the cell surface has been related to many processes of intercellular recognition and adhesion or cell-matrix interactions. Some examples are the

*To whom correspondence should be addressed: Carme de Bolós, Unitat de Biologia Cel-lular i Molecular—IMIM, Dr. Aiguader, 80, 08003 Barcelona, Spain. Tel.: 34 93 221 10 09; Fax: 34 93 221 32 37; E-mail: cbolos@imim.es

interaction between E-selectin and s-Lea and s-Lex antigens associated with glycolipids and glycoproteins expressed on the surface of leukocytes. This interaction promotes the recruitment of leukocytes to the sites of inflammation [21]. Also, α-1,2-fucosylated antigens have been involved in cell motility phenomenon in experimental *in vitro* models [22–24]. Fucosylated structures are also implicated in bacterial adhesion at the first stages of cell infection. In the gastric mucosa, it has been reported that the adhesion of *Helicobacter pylori* to the cells of the superficial epithelium of the gastric mucosa is mediated by the interaction of the bacteria with Leb-expressing cells [25]. The analysis of the bacterial LPS has revealed the presence of Lex and Ley mimicking those in the host to escape from the immune system recognition. Several bacterial glycosyltransferases have been cloned [26] suggesting that these LPS carbohydrates are synthesised by the bacteria [27–29]. In some strains of *H. pylori*, the presence of type 1 Lewis antigens has also been reported [30].

In previous studies, we have described that in the mucus-secreting cells of the superficial epithelium of the gastric mucosa, MUC5AC expression is associated with type 1 Lewis antigens and FUT2 expression whereas in the mucus-secreting cells of the deep glands, MUC6 is detected in association with type 2 Lewis antigens and FUT1. These data suggested that these oligosaccharides could be directly glycosylating the apomucin core [31,32]. To analyse the implication of FUT1 and FUT2 in these patterns of association between apomucin and Lewis antigen expression we selected the HT-29/M3 colon cancer cell line from a characterised panel of cell lines [33] because these cells express MUC1, MUC5AC, type 1 Lewis antigens and FUT2, corresponding to the pattern of expression previously detected in the superficial epithelium of the gastric mucosa [32]. Here, we have analysed the pattern of apomucin glycosylation induced by the expression of human FUT1 cDNA. From our results, the expression of FUT1 induces the synthesis of type 2 antigens, that coimmunoprecipitated with MUC1 and MUC5AC in HT-29/M3 FUT1-transfected cells but not in the parental cell line.

Materials and methods

Cell lines

HT-29/M3 human colon adenocarcinoma cells are derived from the HT-29 colon cancer cell line treated with 10^{-3} M of methotrexate [34]. HT-29/M3 cell monolayer is mainly constituted by absorptive cells with low percentage of mucus-secreting cells (<5%).

Cells were cultured in DMEM supplemented with 10% FCS, 1% L-glutamine, non-essential aminoacids, penicillin and streptomycin (Life Technologies, Inc.) at 37°C in 5% CO_2 atmosphere. Stable transfectants were cultured in DMEM supplemented with 0.5 mg/ml G418 (Life Technologies, Inc.).

Cells were routinely checked for *Mycoplasma* contamination (Stratagene).

Transfection and isolation of stable transfectants

HT-29/M3 cells were transfected with the full-length of human FUT1 cDNA (accession number M35531) inserted in the pCDM7 expression vector (Dr. Lowe, Michigan, USA) [14]. 10 μg of the pCDM7-FUT1 construction and 10 μg of pSV2neo plasmid were co-transfected using lipofectamine as described by the manufacturer (Life Technologies, Inc). Control cells were transfected with 10 μg of the empty vector and 10 μg of pSV2neo. 72 hours later, 0.5 mg/ml G418 was added to the medium to select transfected cells. Individual colonies were isolated using cloning cylinders and expanded. Clones derived from mock-transfected cells were pooled and used as control cells.

RT-PCR

Total RNA was isolated from confluent cells following the Chomczynski-Sacchi method. RNA quality was tested on 0.8% formaldehyde-agarose gels. cDNA was synthesised using 5 μg DNase I-treated RNA with 200 U MMLV-RT. Primers and conditions for the amplification of FUT1 have been described previously [32,35].

Antibodies and immunohistochemical assays

Monoclonal antibodies M8 [36] CLH2 [37], T-218 [38] and 77/180 [31] that recognise MUC1, MUC5AC, Lewis b and Lewis y respectively, were used as undiluted hybridoma supernatant. Monoclonal antibodies BC3 [39] to detect MUC1 and 19-0LE [40] that recognise H type 2 antigen, were used as ascites diluted at 1/250 and 1/1000 respectively. Purified B3 monoclonal antibody to detect Lewis y was used at 10 μg/ml [41]. B12 MoAb (Dr. Castro, Barcelona, Spain) that recognises a synthetic dextran molecule was used as negative control. Rabbit polyclonal serum LUM5.1 [42] detecting MUC5AC and preimmune rabbit serum used as a negative control were diluted at 1/1000. Indirect immunoperoxidase technique was performed on paraffin embedded sections as described [32]. Scoring was performed as an estimated of the whole section and evaluated by two independent observers.

Flow cytometry

Confluent cells were trypsinized and counted. 10^6viable cells were incubated for 30 minutes at 4°C with the primary antibody (M8, T-218 and 77/180 detecting MUC1, Lewis b and Lewis y, respectively) diluted in serum free medium containing 0.5% BSA and 0.2% sodium azide. Cells were rinsed in serum free medium and incubated with the secondary FITC-labelled antibody for 30 minutes at 4°C. After two washes, fluorescent analysis was performed using a FACScan (Becton-Dickinson, CA).

Tumorigenicity

Confluent cells (M3 parental and M3-FUT1 clones) were trypsinized and 10^6 cells suspended in 0.1 ml D-MEM were

injected subcutaneously into nude Balb/c mice. Animals were killed when tumor size was 1 cm^3. Tumors were embedded in paraffin and immunohistochemistry was performed as described [39]. In some cases, a fragment of the subcutaneous tumor was finely minced and plated to culture tumor cells.

Western blotting

Cell lysates were prepared incubating the cells with 50 mM TRIS pH 7.5, 5 mM EDTA, 6 mM guanidinium hydrochloride, 1 mM phenyl methyl sulfonyl fluoride and protease inhibitors for 30 minutes, centrifuged and supernatant was used.

Proteins were reduced and applied on 4% SDS-PAGE running gels and transferred onto nitrocellulose (PROTRAN, Schleicher and Schuell Gmbh, Germany) membranes. In selected cases 6% SDS-PAGE running gels with 3.5% stacking gels were used. Non-specific binding sites were blocked using 5% skim milk in PBS-0.1%Tween. Primary anti-mucin and anti-sugar antibodies were incubated for 1 h in PBS containing 1% BSA. B12 MoAb and pre-immune rabbit serum were used as negative controls diluted 1/5 and 1/1000 respectively. After 3 washes in PBS-0.1%Tween, peroxidase-conjugated secondary antibodies were applied for 1 h. After washing, reaction was revealed by chemiluminiscence using the ECL kit (Amersham Pharmacia Biotech).

In some cases, membrane stripping was performed incubating the membranes in stripping buffer containing 100 mM 2-mercaptoethanol and 2%SDS in 62.5 mM Tris-HCl pH 6.8 at 55°C. The absence of bands after stripping was controlled in all the cases.

Metabolic labelling and immunoprecipitation

6-well postconfluent cultures of HT-29/M3 cells were preincubated with methionine free medium for 30 minutes at 37°C. Pulse labelling was performed with the addition of 10 μCi/ml L-[^{35}S]methionine to the cells and incubated for 1 h. The medium containing the radiolabelled methionine was removed, cells were rinsed with sterile PBS and medium containing 2 mM methionine was added. Cells were incubated at 37°C for 1–24 h. After the corresponding pulse-chase experiment, cells were washed with cold PBS and lysis buffer containing protease inhibitors was applied for 30 minutes. Cell were scraped and centrifuged for 30 minutes. The supernatant was incubated with anti-apomucin antibodies overnight at 4°C. B12 MoAb and pre-immune rabbit serum were used as negative controls for immunoprecipitation. Immunocomplexes were precipitated using immunoprecipitin from *Staphylococus aureus* (Life Technologies, Inc.). The immunoprecipitated proteins were exhaustively washed, reduced and loaded on 6% SDS-PAGE running gels with 3.5% stacking gels for the analysis of MUC1 and 4% gels for MUC5AC. Gels were fixed in 10% methanol and 7% acetic acid, incubated with Amplify (Amersham Pharmacia Biotech) and dried. Films were exposed for 1–4 weeks at −80°C.

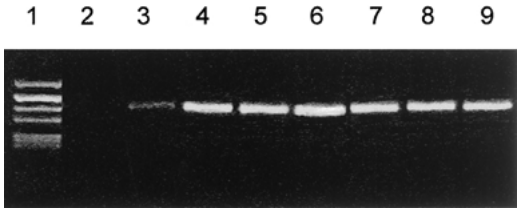


Figure 1. FUT1 mRNA detection by RT-PCR in M3-FUT1 clones. Lane 1: Molecular weight marker, 2: HT-29/M3 parental cells, 3: M3-mock cells, 4: C48, 5: C49, 6: C50, 7: C55, 8: C66 and 9: C81. FUT1 cDNA is not detected in HT-29/M3 parental cells but is slightly expressed in M3-mock transfected cells. C48, C49, C50, C55, C66 and C81 are representative M3-FUT1 clones.

Immunoprecipitated proteins separated by electrophoresis were also transferred onto nitrocellulose membranes. Western blotting was performed as described above.

Results

M3-FUT1 clones display the "de novo" expression of type 2 Lewis antigens

Using RT-PCR, FUT1 mRNA was not detected in HT-29/M3 and slightly expressed in mock-transfected cells. Instead, in M3-FUT1 clones FUT1 mRNA was detected, as shown in Figure 1.

By flow cytometry and immunohistochemistry MUC1 was detected in all the cell lines, and its expression was used as a positive control (Figure 2). Lewis type 1 antigens (Lea, Leb, sLea) were detected in both parental and M3-FUT1 cells by immunohistochemistry and flow cytometry with low levels of expression (20–30% of positive cells) (data not shown). Instead, Lewis type 2 antigens (H type 2 precursor and Ley) were only detected in M3-FUT1 cells (Figure 2), although sLex was only focally detected in parental, mock and FUT1-transfected cells. Clones C50 and C55 were selected because they showed the highest levels of expression of type 2 antigens (H type 2 and Ley), 50% of positive cells by immunohistochemistry (Figure 2) and 80% of positive cells by flow cytometry (Figure 2), and used for further studies.

FUT1 expression does not induce changes in the tumorigenicity of the cells

Parental, mock and FUT1-transfected cells were injected subcutaneously in nude mice (M3 parental $n = 4$, M3 mock $n = 5$, C50 $n = 6$, and C55 $n = 6$) to analyse the implication of type 2 antigens in the formation and growth of tumors. HT-29/M3 cells and mock-transfected cells develop subcutaneous tumors of 1 cm^3 in 5–6 weeks after injection of the tumor cells. M3-FUT1 cells, C50 and C55 clones, showed no significant differences regarding their ability to develop tumors and the size of the tumors obtained. In all the cases, parental and M3-FUT1 cells, develop tumors that are histologically classified as

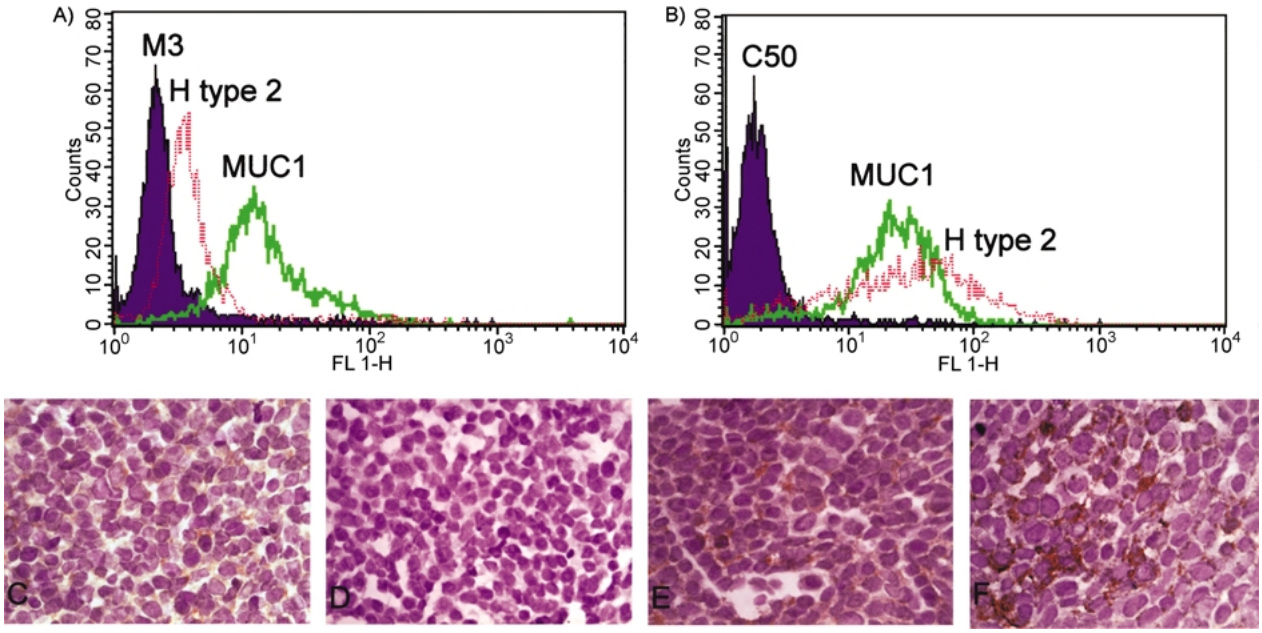
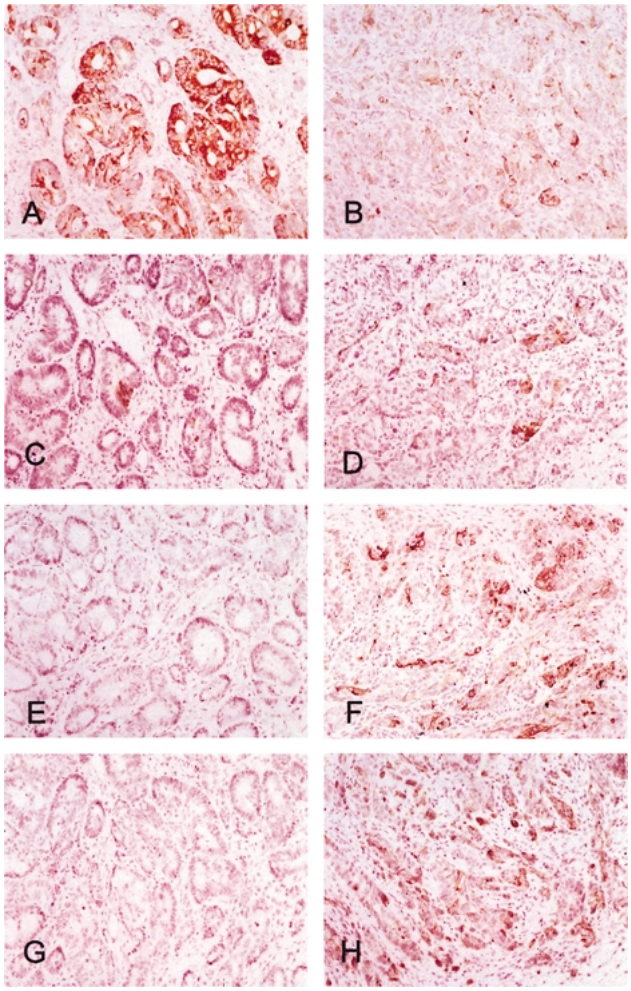


Figure 2. MUC1 and H type 2 antigen detection by flow cytometry (A, B) and immunohistochemistry (C–F) in M3 parental cells (A, C, D) and C50 M3-FUT1 transfected cells (B, E, F). MUC1 is detected in both parental and transfected cells (A, B, C, D) but type 2 antigens, H type 2 (A, B) and Lewis y (E, F) are only detected in M3-FUT1 cells.



adenocarcinoma. The tumors originated from M3 parental cells display a higher degree of differentiation comparing with the tumors from the C50-FUT1 transfected cells.

Apomucin and Lewis antigen expression was analysed by immunohistochemistry and we found that the number of cells expressing both apomucins, MUC1 (data not shown) and MUC5AC (Figure 3), and Lewis antigens, type 1 (data not shown) and type 2 (Figure 3), was increased in subcutaneous tumors probably because mucus-secreting cells had been selected during the growth of the tumors in mice. Also, the expression of MUC5AC increased with the degree of differentiation of the tumor, until 90–100% of positive tumor cells, compared with the low levels of MUC5AC expression in HT-29/M3 parental cells, mock-transfected and transfected cultured cells. The expression of Lewis type 2 antigens was only detected in the FUT1-transfected cells (Figure 3). These tumor cells were cultured and used for further studies. We selected the cell lines M3-4b and C50-6a from mice injected with M3 parental cell line and clone C50 respectively, that keep the patterns of apomucin and Lewis antigen expression from subcutaneous tumors.

Figure 3. Apomucin and Lewis antigen expression analysed by immunohistochemistry on subcutaneous tumors from mice injected with M3 parental cells (A, C, E, G) and C50 M3-FUT1 clone (B, D, F, H). The expression of MUC5AC (A, B) and Lewis b (C, D) is detected in tumors from M3 and C50 cells whereas H type 2 antigen (E, F) and Lewis y (G, H) are only detected in tumors from C50 cells.

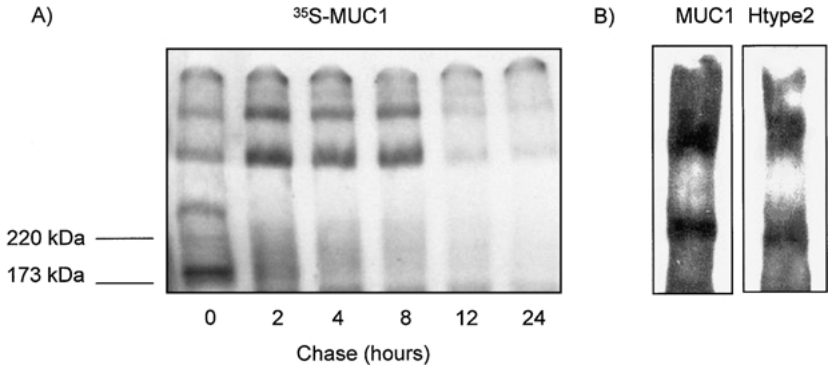


Figure 4. (A) C50-6a FUT1-transfected cells were pulsed with [^{35}S]-methioninechased at different times (0–24 hours) and immunoprecipitated using anti-MUC1 M8 antibodies. Immunocomplexes were run on a 6% running gel with 3.5% stacking gel. Markers (-) indicate the limit between stacking and running gel, and arrowheads indicate the position of 220 and 173 kDa molecular weight markers. The two lower molecular weight bands corresponding to unglycosylated MUC1 are only present in the lane 0. (B) Immunocomplexes from C50-6a cells immunoprecipitated with M8 antibody were run on 4% running gels without stacking gel, transferred onto nitrocellulose and blotted using M8 and 19-0LE antibodies recognising MUC1 and H type 2 antigens respectively. The two high molecular weight bands corresponding to glycosylated MUC1 colocalise with the bands obtained with 19-0LE antibody.

FUT1 directly instructs the glycosylation of MUC1 and MUC5AC apomucin backbone

HT-29/M3 parental cells express MUC1 and MUC5AC whereas other mucins as MUC2, MUC4 and MUC6 were not detected by immunohistochemistry and RT-PCR [40].

Firstly, we labelled the cells with [^{35}S]-methionine and analysed the expression of MUC1 and MUC5AC by immunoprecipitation using specific antibodies (M8 for MUC1 and LUM5.1 for MUC5AC) and developed by autoradiography. For MUC1, in M3-4b cells, we found the presence of two bands of approximately 180 and 250 kDa in 6% SDS-PAGE running gels that may correspond to the two polymorphic precursor forms of apomucin MUC1, as described [43]. In the stacking gel, we determined the presence of two higher molecular weight bands corresponding to the glycosylated forms of the previous precursor unglycosylated proteins. In the chase experiments (2–24 hours), only the glycosylated forms appear. The same results were obtained with C50-6a cells (Figure 4). In any case, we detected bands in the gel after immunoprecipitation with B12 MoAb negative control (data not shown). The MUC1 immunoprecipitates were also run and transferred onto nitrocellulose membranes to assay the coimmunoprecipitation with determined oligosaccharides. From these experiments, we found that MUC1 in M3-4b cells didn't coimmunoprecipitate with any of the type 2 Lewis antigens studied (H type 2 precursor and Ley) (data not shown). In C50-6a cells MUC1 coimmunoprecipitated with H type 2 antigens (Figure 4) and Ley (data not shown) when blotting was performed using 19-0LE and B3 antibodies respectively.

Regarding MUC5AC, after labelling M3-4b cells with [^{35}S]-methionine and immunoprecipitation using LUM5.1 antibodies, we detected a very high molecular weight band in 4% SDS-PAGE running gels that correspond to the MUC5AC apomucin

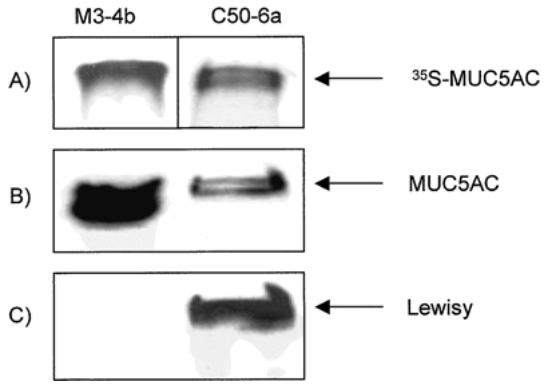


Figure 5. M3-4b and C50-6a FUT1-transfected cells were labelled with [^{35}S]-methionine, homogenised and immunoprecipitation was performed using anti-MUC5AC LUM5.1 antibodies. Immunocomplexes were run on 4% running gels and developed by autoradiography (A). Cell lysates were run and transferred onto nitrocellulose and blotted using LUM5.1 (B). After stripping the membranes, B3 antibody was blotted (C). In these experiments the colocalisation between MUC5AC and type 2 antigens (Ley) is only detected in C50-6a cells.

(Figure 5A and B), as described by Van Klinken [44]. In C50-6a cells, MUC5AC is also detected by autoradiography and western blotting. This high molecular weight band didn't appear when serum from pre-immunized rabbits was used as negative control (data not shown). When the membranes used to detect MUC5AC were stripped and incubated with anti-Lewis antigens antibodies, we found that MUC5AC and type 2 structures (Ley) were codetected in the FUT1-transfected C50-6a cells but not in parental cells, M3-4b, using LUM5.1 and B3 antibodies (Figure 5C). The same results were obtained with 19-0LE antibody detecting H type 2 antibodies in the same membranes (data not shown).

These results indicate that the induced expression of FUT1 determines the association and coimmunoprecipitation of MUC1 and MUC5AC with type 2 Lewis antigens in this *in vitro* model.

Discussion

In this paper we describe changes in the carbohydrate structures associated to MUC1 and MUC5AC as a consequence of FUT1 transfection in HT-29/M3 cells. Until now, there are only few data supporting directly that apomucin glycosylation is specifically instructed by the specific set of glycosyltransferases expressed at the single cell level, and not only directed by the primary sequence of the protein. Previously, we have obtained indirect evidence supporting the first hypothesis [32] from results on gastric carcinogenesis, indicating that the pattern of apomucin glycosylation was not only dependent on the aminoacid sequence but also in the glycosyltransferases expressed in each cell type. Supporting these hypothesis, published data from *in vivo* glycosylation experiments of mucin tandem repeats using chimeric mucins with combinations of TR domains corresponding to different apomucins, report limited influence in this posttranslational process by the presence of these substituted TR domains [45]. Supporting the fact that glycosyltransferases are responsible for the specific pattern of mucin glycosylation, Axelsson demonstrated recently that the neutralisation of pH in the Golgi apparatus causes redistribution of glycosyltransferases and the consequent changes in the glycosylation pattern of mucins [46]. Until now, the published data regarding MUC1 glycosylation are based on the observation that different carbohydrate structures are associated to the apomucin core in pathologies as cancer, as is the association between Ley and MUC1 in ovarian cancer [11]. The differences in the oligosaccharide profiles that bear breast cancer cells in comparison to normal breast epithelial cells were reported by Lloyd [47], and the implication of specific glycosyltransferases in the glycosylation of MUC1 has been recently described by Dalziel, reporting the implication of C2GnT1 and ST3Gal-I enzymes in the O-glycan structure attached to MUC1 protein [48]. Recently, Teatert demonstrated that specific GalNacT are involved in different steps of the sequential process of O-glycosylation using synthetic peptides mimicking the tandem repeat of MUC5AC [49,50]. From our results, we conclude that changes in the pattern of glycosylation of MUC1 and MUC5AC are based on the expression of FUT1 in the HT-29/M3 *in vitro* model, confirming the hypothesis that the expression of specific fucosyltransferases, in this case FUT1, determines the pattern of mucin glycosylation.

The transmembrane mucin MUC1 has been described as an anti-adhesive molecule, and MUC1 overexpression causes inhibition of integrin-mediated cell adhesion to extracellular matrix components [51]. Zhang et al. reported that sLea and Lex were associated to MUC1 in colon cancer cells and that glycosylated MUC1 inhibited the adhesion of leukocyte cell line to cells transfected with E-selectin [52]. MUC1 is also a ligand for ICAM-1 facilitating the binding to the endothelium in the process of metastasis [53] and the interaction of MUC1 with E-cadherin has been demonstrated with anti-adhesive properties [54].

For MUC5AC, the biological functions proposed are related to the formation of the gel that covers the epithelial surface including the ability of oligomerization associated to the cluster of mucins in the 11p15.5 locus [55] and also, the long carbohydrate chains attached to the apomucin backbone are responsible for the protective functions proposed for mucins. In this sense, carbohydrate structures are long and complex in normal tissues and shorter chains are usually related to pathologies such as cancer. Also, MUC5AC protein has been detected associated with pS2 or TFF1 in the superficial mucosa of the stomach [56,57] and this interaction has been demonstrated using the yeast 2-hybrid system [58]. The implication of these small peptides in the renewal of the gastric mucosa after injury may suggest the implication of MUC5AC in this process, at least in the stage of stabilisation of the gel that covers the gastric epithelium [59–62]. Regarding the carbohydrates that have been described to be associated to MUC5AC, Boren reported that the pathogenic bacteria *Helicobacter pylori* colonise the gastric mucosa through the binding of fucosylated structures, specifically Leb [25]. As we described in a previous report [32], MUC5AC and Leb show overlapping patterns of expression in the mucus-secreting cells of the superficial epithelium of the stomach and that correlates with the pattern of expression of the α-1,2-fucosyltransferase FUT2. With the present demonstration that type 2 antigens are directly attached to MUC5AC using a biochemical approach, it might be possible the implication of MUC5AC in the pathogenesis of *H. pylori* in the gastric mucosa. Supporting this hypothesis, Van den Brink reported recently the colocalisation of MUC5AC with *H.pylori* in the gastric mucosa [63].

From our results, the transfection of FUT1 and the expression of type 2 antigens in HT-29/M3 cells didn't correlate with changes in the expression of other carbohydrates as type 1 antigens. Sepp [64] reported an increase in the expression of H antigen and the *de novo* expression of Ley after the transfection of PLECT porcine endothelial cells with human FUT1, together with a reduction in the levels of expression of porcine α-galactose antigen and sialylated structures, suggesting the competition between α-1,2-fucosyltransferases, α-1,3-galactosyltransferases and sialyltransferases, as the activities of theses enzymes were not altered. Previously, Sharma [65] demonstrated the competition *in vitro* between α-1,3-galactosyltransferases and α-1,2-fucosyltransferases for the same glycoprotein substrate using CHO cells. In the case of HT-29 cell line, it has been reported the expression of α-2,3-ST and four different α-1,3/1,4-FucT by RT-PCR that give rise to Lea and Lex and the sialylated structures sLea and sLex [66]. Recently, Gouyer et al. reported the expression of several sialyltransferases in HT-29 cells by multiplex RT-PCR and their

corresponding enzymatic activity [67] but there is no data reporting the pattern of sialyltransferase and fucosyltransferase expression on HT-29/M3 cells. In these cells, we have also found low levels of expression of FUT2 and FUT3 giving rise to the expression of type 1 Lewis antigens at low levels [33]. Furthermore, we haven't found differences in the expression of sialylated structures in the transfected cells when compared with the low levels of expression detected in the parental cell line. Altogether, the low levels of carbohydrates synthesised in HT-29/M3 cells make it difficult to find slight differences in their levels of expression.

In conclusion, our results using the HT-29/M3-FUT1 *in vitro* model indicate that the specific glycosylation patterns displayed by the mucin molecules MUC1 and MUC5AC is instructed, in the case of fucosylated structures, by the specific set of glycosyltransferases expressed in the cell and not by the primary aminoacid sequence. As fucosylated carbohydrates has been implicated in the bacterial colonisation of gastric epithelial cells, the *in vitro* models synthesising these epitopes may be useful to better analyse the processes that occur in the gastric epithelium during the colonisation process.

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