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Fucosylation in cystic fibrosis airway epithelial cells

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Altered glycosylation is a phenotypic characteristic of cystic fibrosis (CF), and some of the alterations are summarized. The lungs are the site of the lethal pathology of the disease. Therefore, two of the characteristics were examined in CF and non-CF immortalized airway epithelial cell lines (AEC). The activity of α-L-fucosidase was elevated (280%) in CF AEC when compared with non-CF AEC, whereas the activities of the other lysosomal enzymes which were examined were similar in both cell types. α-L-Fucosidase activity was transiently increased in the non-CF cells after treatment with Brefeldin A (BFA) for 6 h. Thus BFA caused the normal cells to express a phenotypic characteristic of CF. Glycopeptides from the CF and non-CF AECs metabolically labeled with L-[^{3}H]fucose were examined for binding to lentil lectin–Sepharose. A higher percentage of CF glycopeptides bound to lentil lectin, 43% compared with 23% for non-CF control. In addition, a higher percentage of CF glycopeptides were bound tightly to lentil lectin and required 0.2 M α-methylmannoside to be eluted. This species of tightly bound glycopeptides increased dramatically to 77% from 46% when the CF AEC were treated with BFA. In contrast, the non-CF cell glycopeptides had a minor decrease in tightly bound glycopeptides to 26% from 33% after BFA treatment. Thus, the CF AEC showed fucosylation alterations observed previously for other CF cells and tissue.

Keywords: airway epithelial cells, CFTR, cystic fibrosis, α-L-fucosidase, fucosylation

Introduction

Cystic fibrosis (CF) is the most common lethal genetic disease among the Caucasian population [1]. The CF gene has been cloned, however the function of the protein product of the gene, called the cystic fibrosis transmembrane conductance regulator (CFTR), has not been fully delineated [2]. CFTR is a membrane glycoprotein and is known to function as a Cl^- channel [2], although it is not clear how a decreased chloride conductance alone can bring about the diverse pathology observed in CF.

Glycosylation alterations are among the phenotypic characteristics of CF, and a representative summary is presented in Table 1. A number of these studies reflect the altered compositions of CF mucins, which are often expressed as altered ratios of fucose to sialic acid, as originally described by Dische *et al.* [3]. The membrane glycoproteins of skin fibroblasts from CF patients have also been shown to contain an altered ratio of fucose to sialic acid when compared with normal fibroblasts [4]. In addition, a higher percentage of CF than of non-CF fibroblast glycoproteins bound tightly to lentil lectin–Sepharose. Lentil lectin–Sepharose binds glycoproteins with fucosyl residues linked α-1,6 to the core *N*-acetylglucosamine (GlcNAc). These findings were verified with the use of high-resolution nuclear magnetic resonance (NMR) spectroscopy [5]. The CF glycoproteins, when examined by ^{1}H-NMR spectroscopy, contained, in addition to more fucosyl residues in α-1,6 linkage, fucosyl residues in α-1,3 linkage to an antennary

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Table 1. Abnormal glycosylation in CF[a]

Source	Fraction examined	Alterations in CF[b]	Reference
Duodenum	Mucins	↑ Ratio Fuc:NeuAc	3
Rectum	Mucins	↑ Ratio Fuc:NeuAc	25
Serum	Heparin-bound glycoproteins	↑ Fuc; ↓ NeuAc	26
Plasma	α_2-Macroglobulin	↓ NeuAc	27
Meconium	Mucus glycoproteins	↑ Fuc	28
Fibroblasts	Membrane glycopeptides	↑ Fuc	29
Lung	Mucins	Novel oligosaccharide sequences	30
Fibroblasts	Medium	Heparin bound; altered turnover	31
Intestine	Mucins	↑ Ratio Fuc:NeuAc	32
Serum	Immunoglobulin G	↓ NeuAc and Gal	33
Lung	Mucins	NeuAc and Fuc novel oligosaccharide	34
Fibroblasts	Peripheral glycopeptides	↑ Fuc; binding to Fuc lectin, lentil	4
Nasal epithelial cells	Glycoconjugates	↑ Sulfation	35
Fibroblasts	Peripheral glycopeptides	↑ Fucα-1,6 and α-1,3-linked	5
Transfected COS cells[c]	CFTR	Retained in ER	19
Small intenstine	Mucins	↑ Binding to Fuc lectin, LTG	36
Fibroblasts	*N*-linked glycopeptides	↑ Ratio Fuc:Gal	37
Immortalized AEC[d]	Glycoproteins	↓ Sialylation	8
Salivary gland	Mucins	↑ NeuAc; binding of *Pseudomonas*	38
Serum and meconium	α_1-antitrypsin	↑ Binding to lectins ConA and WGA	39
CF-PAC cells[e]	α-2,6-NeuAc transferase	↓ Activity[f]	46
Transfected C127 cells[g]	Cell histochemistry	↓ Sialylation	41
Lung	Mucins	↑ Binding of *Pseudomonas*	42

[a]Selected summary.
[b] ↑ increased; ↓ decreased.
[c]Monkey kidney cell line, COS-7 transfected with ΔF508 CFTR.
[d]Airway epithelial cells.
[e]Pancreatic tumor of CF patient.
[f]Elevated on correction with CFTR.
[g]Mouse mammary tumor cell line, C127 transfected with ΔF508 CFTR.

GlcNAc. The control glycopeptides contained less Fuc(α1–6) and no Fuc(α1–3) [5].

In another series of experiments, α-L-fucosidase was shown to have increased activity in skin fibroblasts from CF patients when compared with that of control fibroblasts [6]. At the same time, the activities of eight other lysosomal enzymes were not elevated. Extracellular α-L-fucosidase activity as measured in CF serum was decreased when compared with controls [7]. Again, activities of other hydrolases examined did not differ from controls.

The significance of the structural alterations (Table 1) as well as the elevated α-L-fucosidase activity could be accounted for by the influence of CFTR on trafficking within the CF cell. Indeed, it has been proposed by Barasch *et al.* [8] that altered Cl^- conductance within the intracellular vesicles of CF cells affects glycoprotein processing. This could provide a unifying hypothesis for the observed altered glycosylation (Table 1) and the fact that CFTR serves as a Cl^- channel which is altered in CF cells [2].

Since airway cells are the major site of the lethal pathology in CF, it was of interest to examine CF airway epithelial cells for some of the phenotypic characteristics previously described in the skin fibroblasts and other cell types and tissues. We report here two of these characteristics, the activity of α-L-fucosidase and the binding of ^{3}H-labeled glycopeptides to lentil lectin–Sepharose.

Material and methods

Cell culture and harvest

Two airway epithelial cell lines were used for these studies. Normal airway epithelial cells, BEAS-2B, were immortalized by adeno/SV40 virus and obtained from Dr J. F. Lechner, NIH [9]. BEAS-2B cells were grown as described previously [10] with the exception that they were passaged once per

week at 2×10^6 cells per 75-cm^2 flask coated with 25% fibronectin. CF airway epithelial cells, CF/T-43, were obtained by immortalizing nasal epithelial cells from a CF patient homozygous for the most common mutation, ΔF508 [11]. These cells were immortalized with SV40 T antigen and obtained from Dr J. R. Yankaskas, University of North Carolina. CF/T-43 cells were cultured as described previously [11] and passaged once per week at 2×10^6 cells per 75-cm^2 flask. When radioactive glycopeptides were prepared, the cells were metabolically labeled (10 μCi per 75 cm^2 flask) with L-[5,6-^{3}H]fucose (60 Ci/mmol; NEN) for 24 h prior to harvest. For some experiments the cells were treated with 1.3 μg of Brefeldin A (BFA; Epicentre Technologies, Madison, WI, USA) per ml of culture medium for 1–24 h as specified. The two cell lines were harvested on days 5 or 6 with controlled trypsinization releasing the membrane glycopeptides and maintaining the viability of the cells [4, 5]. Aliquots were taken for cell count and protein determination, using bovine serum albumin as standard [12], and the cell pellets were frozen at −25°C until used. The cell lines show Cl^- conductance characteristic of airway epithelial cells from CF (CF/T-43) or non-CF (BEAS-2B) origin. The genotype of the cells in relationship to the CF mutation ΔF508 was verified by Dr G. Diamond, Division of Genetics.

Extraction of lysosomal enzymes

The cell pellets were resuspended at 2.3×10^7 cells per ml of 0.1% Triton X-100 and then sonicated four times in ice-cold water for 8 s each, alternated with chilling on ice for 12 s. The homogenate was centrifuged at 1120 ***g*** for 10 min and then the supernatant solution assayed for the activity of α-L-fucosidase, acid phosphatase, β-D-galactosidase and β-hexosaminidase, using 4-methylumbelliferyl (4-MU) derivatives [6].

Lysosomal enzyme assays

Aliquots of the extracts were brought to a total volume of 100 μl with 0.1% Triton X-100 and held for 15 min at 37°C. The reaction was started by adding 100 μl of the appropriate 4-MU substrate (Sigma). 4-MU-*N*-acetyl-β-D-glucosaminopyranoside, 4-MU-β-D-galactopyranoside and 4-MU-phosphate were in 1.3 mM citrate buffer, pH 4.3, and 4 MU-fucopyranoside was in citrate buffer, pH 5.8. Unless specified, the samples were incubated at 37°C for 1 h. The reaction was stopped with 1.8 ml of 0.125 M glycine-carbonate buffer, pH 10. The enzyme activity was measured by the 4-MU released from the substrate with an Aminco SPF spectrofluorimeter set at excitation 360 mm and emission 450 mm.

Quantitation of α-L-fucosidase

α-L-Fucosidase protein was detected in Triton extracts of CF and non-CF cells by precipitation with a polyclonal anti-fucosidase antiserum [13]. Quantitation of enzyme protein was measured by enzyme-linked immunosorbent assay (ELISA). The specificity and quantitation of this assay have been described previously [13].

Partial characterization of the membrane glycoproteins

The large membrane glycoproteins released by trypsin from the cells metabolically labeled with L-[^{3}H]fucose were desalted on Biogel P-2 and separated on lentil lectin–Sepharose (Pharmacia), collecting 2-ml fractions. The ^{3}H-labeled glycopeptides which bound to lentil lectin–Sepharose were eluted with 10 mM and 0.2 M α-methyl mannoside, and counted in a liquid scintillation counter. In some experiments the ^{3}H-labeled glycopeptides which were desalted on Biogel P-2 were treated with α-1,3/4-fucosidase from almonds as described previously [14]. [^{3}H]Fucose which was released from the treated glycopeptides was detected by scintillation counting after paper chromatography in pyridine–ethyl acetate–water (4:10:3) as described previously [15].

Results

Lysosomal enzyme activity

The activity of several lysosomal enzymes from CF airway epithelial cells from a patient homozygous for the most common mutation, ΔF508 (CF/T-43), were compared with non-CF airway epithelial cells (BEAS-2B). The activities of α-L-fucosidase, β-hexosaminidase, β-galactosidase and acid phosphatase are summarized in Figure 1, where they are expressed as percentage of control with the mean and SD. Only the activity of α-L-fucosidase was increased significantly (280%) in CF/T-43 cells when compared with BEAS-2B cells. The other enzymes were similar or slightly increased.

Using 4-MU-fucosidase as substrate, the activity of α-L-fucosidase was linear with cell number for both CF/T-43 and BEAS-2B cells. Therefore, a limiting factor in the utilization of the substrate by BEAS-2B cells was not present. In order to verify further that the activity of α-L-fucosidase was not

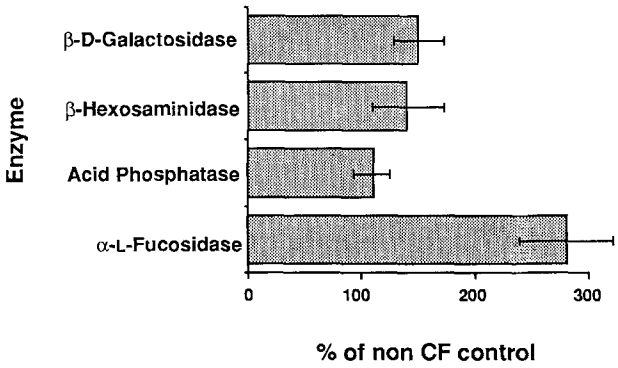

Figure 1. Activity of lysosomal enzymes of airway epithelial cells. The activity of each enzyme from CF/T-43 cells was calculated per cell and expressed as percentage of non-CF control (BEAS-2B) for the indicated enzymes. The enzyme activity in the control (BEAS-2B) cells of α-L-fucosidase, acid phosphatase, β-hexosaminidase and β-galactosidase was 112, 504, 2410 and 342 nmol of 4-MU released/10^7 cells/h respectively. Standard deviation (⊢—⊣) was calculated by the t distribution method; $n = 10$ different experiments for all enzymes with the exception of β-galactosidase ($n = 4$).

limited by the assay, two concentrations of cells were incubated with the substrate for up to 98 h. Again α-L-fucosidase activity became limiting only with time and protein concentration (Figure 2). At 98 h the substrate was utilized by all the extracts with the exception of that from the lower number (5.75×10^5) of BEAS-2B cells. Thus, the activity of α-L-fucosidase was linear with time and protein concentration.

The elevated activity of α-L-fucosidase in the CF cells was shown to correspond to an actual elevation in enzyme protein (37 ng compared with 14 ng per cell for CF/T-43 and BEAS-2B cells respectively; $n = 3$). Within the three matched sets the specific activity varied from 0 to twofold (0.11–0.23 nmol/mg protein/min). In these cases, BEAS-2B α-L-fucosidase gave the higher specific activity; therefore, on the basis of the quantitative ELISA assay [13], it was concluded that the CF/T-43 cells contained more α-L-fucosidase than the BEAS cells.

Lysososmal enzyme activity in the presence of BFA

BFA, a fungal metabolite, has been shown to disrupt traffic between the endoplasmic reticulum (ER) and the Golgi apparatus [16], resulting in altered glycosylation [17, 18]. Since it has been proposed that a defective CFTR may remain in the ER [19], it was of interest to determine if BFA invoked a greater difference in normal cells than in CF cells. For this reason, CF/T-43 and BEAS-2B

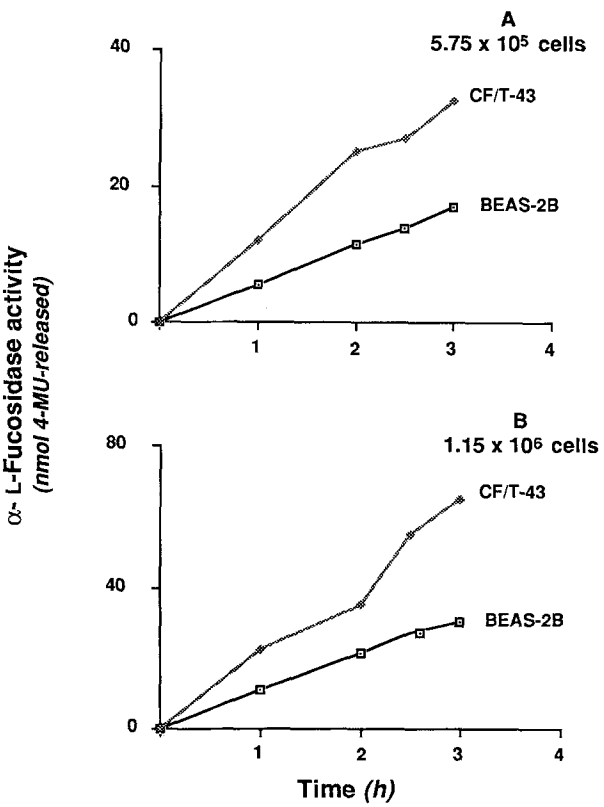

Figure 2. Activity of α-L-fucosidase over extended time. Extracts from (A) 5.75×10^5 and (B) 1.15×10^6 airway epithelial cells were incubated with 1.29 mM 4-MU-fucoside for a total of 98 h. The enzyme activity is shown to 3 h. The activity of α-L-fucosidase at 98 h was 650 nmol of 4-MU released for all extracts, with the exception of (A) BEAS-2B extract, which was 465 nmol.

cells were treated with 1.3 μg of BFA per ml of culture medium for 1, 3, 6 and 24 h, and examined for the activity of α-L-fucosidase and three other lysosomal enzymes. It was found that the enzymes from the treated cells remained similar to that of the non-treated cells for both cell types with the exception of a 6 h treatment (Figure 3). The apparent increase in enzyme activities of the BFA-treated BEAS-2B cells at 6 h was repeated in two separate experiments. Marked increases in enzyme activity were observed for α-L-fucosidase (146%) and acid phosphatase (163%). At 3 h the morphological appearance on both cell types in culture had changed from an epithelial appearance to more rounded shape. This appearance persisted to the termination of the treatment at 24 h.

Membrane glycopeptides from [^{3}H]fucose-labeled cells

The second phenotypic characteristic observed in CF fibroblasts, that is the binding of the mem-

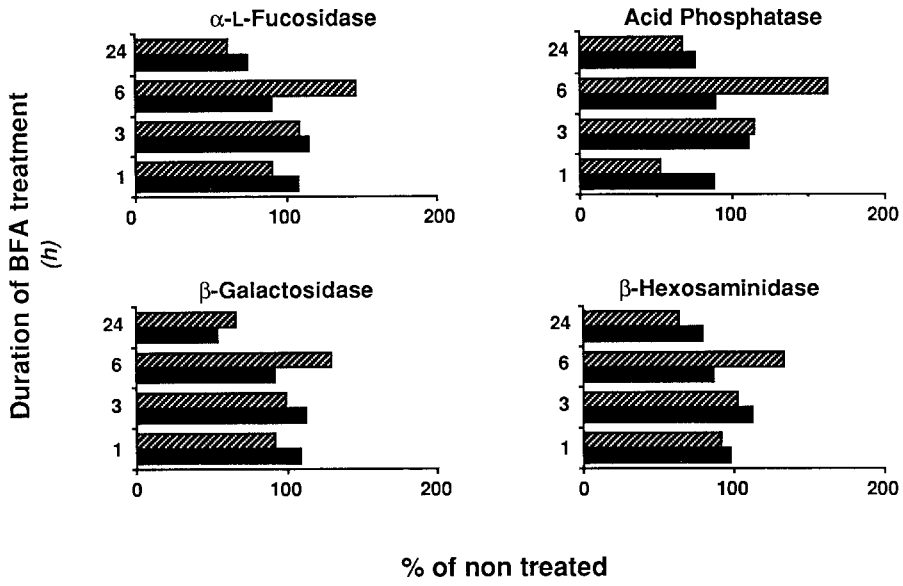

Figure 3. Activity of lysosomal enzymes after treatment of airway epithelial cells with BFA. The cells were treated with 1.3 μg of BFA per ml of medium for the designated times. The results are expressed as percentage of the non-treated. The activity of α-L-fucosidase in the control (non-treated) BEAS-2B cells at 1, 3, 6, and 24 h was 106, 99, 93, and 104 nmol of 4-MU released/10^7 cell/h respectively. The activities of the other enzymes are given in Figure 1. ■, CF/T-43, ▨, BEAS-2B cells; $n = 2$.

brane glycopeptides to lentil lectin–Sepharose [4], was examined in the airway cells. Membrane glycopeptides were obtained from CF/T-43 and BEAS-2B cells metabolically labeled with L-[^{3}H]fucose after prior treatment with or without BFA for 4 h. The ^{3}H-labeled glycopeptides were desalted and examined on lentil lectin–Sepharose. The ^{3}H-labeled glycopeptides from CF/T-43 cells had approximately 20% more of the total radioactivity bound to lentil lectin–Sepharose than those of BEAS-2B cells (43 and 23%, respectively). CF/T-43 had 10% more which bound tightly and were eluted with 0.2 M α-methylmannoside (Table 2). Most interesting was the redistribution of glycopeptide binding when the cells were treated with BFA. The total percentage of ^{3}H-labeled glycopeptides from CF/T-43 cells which now bound tightly to lentil lectin–Sepharose was increased to 77%, whereas the comparable total and tight binding for BEAS-2B cells slightly decreased (Table 2). Thus, the ^{3}H-labeled glycopeptides from the CF cells were significantly different from those of the

Table 2. Binding of ^{3}H-labeled glycopeptides (percentage of ^{3}H radioactivity) to lentil lectin–Sepharose[a]

Lentil lectin–Sepharose bound fraction	Brefeldin A			
	Treated		Non-treated	
	CF/T-43	BEAS-2B	CF/T-43	BEAS-2B
Total bound	42	15	43	23
Eluted with α-methylmannoside				
10 mM	23	74	54	68
0.2 M	77	26	46	33

[a]Cells were treated with Brefeldin A and metabolically labeled with L-[^{3}H]fucose. ^{3}H-labeled glycopeptides were removed from the cells with trypsin and after desalting were chromatographed on lentil lectin–Sepharose.

non-CF cells. The difference observed previously in the CF fibroblast glycopeptides (Table 1; ref. 4) was markedly amplified by BFA treatment.

The membrane glycopeptides from the CF cells which were BFA treated were examined for the presence of fucosyl residues in α-1,3 linkage to an antennary GlcNAc. The almond fucosidase specific for these fucosyl residues released 12% of the [^{3}H]fucose from the glycopeptides of the BFA-treated cells.

Discussion

The striking elevation of α-L-fucosidase activity and enzyme protein in CF airway epithelial cells which are homozygous for the common ΔF508 mutation when compared with non-CF airway epithelial cells leads to the suggestion that CFTR may play a role in the regulation of glycoprotein processing and/or secretion. Increased activity of α-L-fucosidase has now been shown in two cell types, fibroblasts [6] and airway epithelial cells, from CF patients when compared with non-CF cells (Figure 1). The mechanism of the increased activity is not clear, however it is interesting that this enzyme is not subject to proteolytic processing as reported for other lysosomal enzymes [20].

Eight age-, race- and sex-matched sets of fibroblast cell lines were originally examined and showed increased activity of α-L-fucosidase in the CF cells [6]. In this current study only one matched set of airway epithelial cell lines was examined for lysosomal enzymes since other cell lines which grow with similar characteristics are not as yet available. Recently, another characterized set of CF and non-CF airway epithelial cells have been reported [21] so it will be interesting to examine the α-L-fucosidase activity in these cells.

The difference between the CF glycopeptides and non-CF glycopeptides in regard to fucose metabolism was shown previously by the increased binding to lentil lectin-Sepharose of the CF fibroblast glycopeptides [4]. CF airway cell glycopeptides showed similar binding characteristics, and these glycopeptide species were markedly amplified by BFA treatment (Table 2). BFA increased the percentage of [^{3}H]fucose glycopeptides which bound tightly to lentil lectin–Sepharose in that they were eluted with 0.2 M α-methylmannoside. BFA has been shown to cause an increase in biantennary complex-type glycans, which are undersialylated but core fucosylated [18]. As a result of BFA treatment, the *cis*, medial and *trans* Golgi are disrupted and pattern as characteristic of the ER [16]. During this process, the enzymes of the *cis* and medial Golgi are thought to modify the oligosaccharide processing of newly synthesized glycoproteins in the ER, where they are retained [17]. Using endothelial cells, Sampath *et al.* [22] noted an increase in core fucosylation as measured by pea lectin binding of the oligosaccharides after BFA treatment. In addition, there was no branch fucosylation. In the case of CF/T-43 cells, α-1,3-fucosyl residues were detected on the antennary GlcNAc in the BFA-treated cells in addition to increased core fucosylation as measured by binding to lentil lectin (Table 2). It is not known where α-1,3-fucosylation takes place, however the known dispersion of the Golgi by BFA suggests that CF cells may be more sensitive to this disruption than non-CF cells.

Although the general α-L-fucosidase was increased in the CF airway epithelial cells, BFA treatment did not cause a change in α-L-fucosidase activity. The effect of BFA on the lysosomes appears to be marginal [23], although recent evidence shows that retrograde traffic between the lysosomes and endosomes could be disrupted by BFA [24]. The other interesting fact was the transient increase in the general α-L-fucosidase and acid phosphatase activities when the BEAS-2B cells were treated with BFA for 6 h (Figure 5). In the presence of BFA the non-CF cells acquired a phenotypic expression of CF.

It has been proposed that the defective function of CFTR, the protein product of the CF gene, could affect the pH of intracellular vesicles [8]. This in turn could account for the altered regulation of glycoprotein processing and the resulting altered glycosylation which is characteristic of the CF phenotype as reported here for the airway cells and by others as summarized in Table 1. A full understanding of the function and regulation of CFTR may provide further insight into the regulation of glycoprotein processing.

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References

1. Scanlin TF. Cystic fibrosis. In: Fishman AP, eds. *Pulmonary Diseases and Disorders*, 2nd edn. New York: McGraw-Hill, 1988; 1273–94.
2. Collins FS. Cystic fibrosis: molecular biology and therapeutic implications. *Science* 1992: **256**; 774–9.
3. Dische Z, di Sant'Agnese P, Pallavicini C, Youlos J. Composition of mucoprotein fractions from duodenal fluid of patients with cystic fibrosis of the pancreas and from controls. *Pediatrics* 1959: **24**; 74–91.
4. Scanlin TF, Wang Y-M, Glick MC. Altered fucosylation of membrane glycoproteins from cystic fibrosis fibroblasts. *Pediatr Res* 1985: **19**; 368–74.
5. Wang YM, Hare TR, Won B, *et al.* Additional fucosyl residues on membrane glycoproteins but not a secreted glycoprotein from cystic fibrosis fibroblasts. *Clin Chim Acta* 1990: **188**; 193–210.
6. Scanlin TF, Matacic SS, Pace M, Santer UV, Glick MC. Abnormal distribution of α-*L*-fucosidase in cystic fibrosis: increased activity in skin fibroblasts. *Biochem Biophys Res Commun* 1977: **79**; 869–75.
7. Scanlin TF, Matacic SS, Glick MC. Abnormal distribution of α-L-fucosidase in cystic fibrosis: decreased activity in serum. *Clin Chim Acta* 1979: **91**; 197–202.
8. Barasch J, Kiss B, Prince A, *et al.* Defective acidification intracellular organelles in cystic fibrosis. *Nature* 1991: **352**; 70–3.
9. Reddell RR, Ke Y, Gerwin BL, *et al.* Transformation of human bronchial epithelial cells by infection with SV-40 or adenovirus-12 SV40 hybrid virus, or transfection via strontium phosphate coprecipitation with a plasmid containing SV40 early region genes. *Cancer Res* 1988: **48**; 1904–9.
10. Pfeifer AMA, Mark GE, Malan-Shibley L, *et al.* Cooperation of C-raf-I and C-myc protooncogenes in the neoplastic transformation of simian virus 40 large tumor antigen-immortalized human bronchial epithelial cells. *Proc Natl Acad Sci USA* 1989: **86**; 10075–9.
11. Jetten AM, Yankaskas JR, Stutts MJ, Willumsen NJ, Boucher RC. Persistence of abnormal chloride conductance regulation in transformed cystic fibrosis epithelia. *Science* 1989: **244**; 1472–5.
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951: **193**; 265–75.
13. DiCioccio RA, Barlow JJ, Matta KL. Specific activity of α-L-fucosidase in sera with phenotypes of either low, intermediate, or high total enzyme activity and in a fucosidosis serum. *Biochem Genet* 1986: **24**; 115–30.
14. Giuntoli II R, Stoykova LI, Gillies DRB, Glick MC. Expression of GDP-L-Fuc: Gal(β1–4) GlcNAc-R (Fuc to GlcNAc) α-1,3-fucosyltransferase and its relationship to glycoprotein structure in a human erythroleukemia cell line, HEL. *Eur J Biochem* 1994: **225**: 159–66.
15. Foster CS, Gillies DRB, Glick MC. Purification and characterization of GDP-L-Fuc-N-acetyl-β-D-glucosaminide $\alpha 1 \rightarrow$ 3fucosyltransfrase from human neuroblastoma cells. Unusual substrate specificities of the tumor enzyme. *J Biol Chem* 1991: **266**; 3526–31.
16. Lippincott-Schwartz J, Yuan LC, Bonifacino JS, Klausner RD. Rapid redistribution of Golgi proteins. *Cell* 1989: **56**; 801–13.
17. Lippincott-Schwartz J, Yuan L, Tipper C, *et al.* Brefeldin A's effects on endosomes, lysosomes and the TGN suggest a general mechanism for regulating organelle structure and membrane traffic. *Cell* 1991: **67**; 601–16.
18. Chawla D, Hughes RC. Effects of Brefeldin A on oligosaccharide processing. *Biochem J* 1991: **279**; 159–65.
19. Cheng SH, Gregory RS, Marshall J, *et al.* Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. *Cell* 1990: **63**; 827–34.
20. Leibold DM, Robinson CA, Scanlin TF, Glick MC. Lack of proteolytic processing of α-L-fucosidase in human skin fibroblasts. *J Cell Physiol* 1988: **137**; 411–20.
21. Olsen JC, Johnson LG, Stutts MJ, *et al.* Correction of the apical membrane chloride permeability defect in polarized cystic fibrosis airway epithelia following retroviral-mediated gene transfer. *Hum Gene Ther* 1992: **3**; 253–66.
22. Sampath D, Varki A, Freeze HH. The spectrum of incomplete *N*-linked oligosaccharide synthesized by endothelial cells in the presence of Brefeldin A. *J Biol Chem* 1992: **267**; 4440–55.
23. Strous GJ, van Kerkhof P, van Meer G, Rinjboutt S, Stoorvogel W. Differential effects of Brefeldin A on transport of secretory and lysosomal proteins. *J Biol Chem* 1993: **268**; 2341–7.
24. Jahraus A, Storrie B, Griffiths G, Desjardins M. Evidence for retrograde traffic between terminal lysosomes and the prelysosomal/late endosome compartment. *J Cell Sci* 1994: **107**; 145–57.
25. Roelfs RE, Gibbs GE, Griffin GD. The composition of rectal mucus in cystic fibrosis. *Am J Dis Child* 1967: **113**; 419–21.
26. Pearson RD, Lubin AH. Increased heparin binding in cystic fibrosis: a reflection of altered glycoprotein biosynthesis? *Pediatr Res* 1979: **13**; 834–40.
27. Ben-Yoseph Y, Defranco CL, Nadler HL. Decreased sialic acid and altered binding to lectins of purified α_2-macroglobulin from patients with cystic fibrosis. *Clin Chim Acta* 1979: **99**; 31–35.
28. Clamp JR, Gough M. Study of the oligosaccharide units from mucus glycoproteins of meconium from normal infants and from cases of cystic fibrosis with meconium ileus. *Clin Sci* 1979: **57**; 445–51.

29. Scanlin TF, Glick MC. Dynamic and structural aspects of cystic fibrosis fibroblast membranes. In: Sturgess JM, ed. *Perspectives in Cystic Fibrosis*, Proceedings, 8th International Cystic Fibrosis Congress. Toronto: Canadian Cystic Fibrosis Foundation, 1980; pp. 44–57.

30. van Halbeek H, Dorland L, Vliegenthart JFG, *et al.* Primary-structure determination of fourteen neutral oligosaccharides derived from bronchial-mucus glycoproteins of patients suffering from cystic fibrosis, employing 500-MHz ^{1}H-NMR spectroscopy. *Eur J Biochem* 1982: **127**; 7–20.

31. Scanlin TF, Voynow JA, Thomas EJ, Glick MC. Glycoproteins in culture medium: a comparison from cystic fibrosis and control skin fibroblasts. *Biochemistry* 1982: **21**; 491–7.

32. Wesley A, Forstner J, Qureshi R, Mantle M, Forstner G. Human intestinal mucin in cystic fibrosis. *Pediatr Res* 1983: **17**; 65–9.

33. Margolies R, Boat T. The carbohydrate content of IgG from patients with cystic fibrosis. *Pediatr Res* 1983: **17**; 931–5.

34. Lamblin G, Boersma A, Klein A, Roussel P. Primary structure determination of five sialylated oligosaccharides derived from bronchial mucus glycoproteins of patients suffering from cystic fibrosis. *J Biol Chem* 1984: **259**; 9051–8.

35. Cheng PW, Boat TF, Cranfill K, Yankaskas JR, Boucher RC. Increased sulfation of glycoconjugates by cultured nasal epithelial cells from patients with cystic fibrosis. *J Clin Invest* 1989: **84**; 68–72.

36. Thiru S, Devereux G, King A. Abnormal fucosylation of ileal mucus in cystic fibrosis. I. A histochemical study using peroxidase labelled lectins. *J Clin Pathol* 1990: **43**; 1014–18.

37. Bertrand F, Hermelin B, Paul A, Picard J. Pericellular glycoconjugates of cultured fibroblasts from control and cystic fibrosis patients. *Int J Biochem* 1991: **23**; 51–7.

38. Carnoy C, Ramphal R, Scharfman A, *et al.* Altered carbohydrate composition of salivary mucins from patients with cystic fibrosis and the adhesion of *Pseudomonas aeruginosa*. *Am J Respir Cell Mol Biol* 1993: **9**; 323–34.

39. Duthel S, Revol A. Glycan microheterogeneity of α_1-antitrypsin in serum and meconium from normal and cystic fibrosis patients by crossed immunoaffinoelectrophoresis with different lectins (Con A, LCA, WGA). *Clin Chim Acta* 1993: **215**; 173–87.

40. Barasch J, Al-Awqati Q. Defective acidification of the biosynthetic pathway in cystic fibrosis. *J Cell Sci* 1993: **17**; 229–33.

41. Dosanjh A, Lencer W, Brown D, Ausiello DA, Stow JL. Heterologous expression of ΔF508 CFTR results in decreased sialylation of membrane glycoconjugates. *Am J Physiol* 1994: **35**; C360–6.

42. Devaraj N, Sheykhnazari M, Warren WS, Bhavanandan VP. Differential binding of *Pseudomonas aeruginosa* to normal and cystic fibrosis trachebronchial mucins. *Glycobiology* 1994: **4**; 307–16.

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