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Commentary

Glycosylation and the cystic fibrosis transmembrane conductance regulator

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Abstract

The cystic fibrosis transmembrane conductance regulator (CFTR) has been known for the past 11 years to be a membrane glycoprotein with chloride channel activity. Only recently has the glycosylation of CFTR been examined in detail, by O'Riordan *et al* in *Glycobiology*. Using cells that overexpress wild-type (wt)CFTR, the presence of polylactosamine was noted on the fully glycosylated form of CFTR. In the present commentary the results of that work are discussed in relation to the glycosylation phenotype of cystic fibrosis (CF), and the cellular localization and processing of ΔF508 CFTR. The significance of the glycosylation will be known when endogenous CFTR from primary human tissue is examined.

Keywords: ΔF508 cystic fibrosis transmembrane conductance regulator (CFTR), oligosaccharides, polylactosamine

Introduction

Interest in glycosylation has been rekindled in the field of CF research since the identification of the CF gene, which encodes the CFTR membrane glycoprotein [1]. The renewed interest has been stimulated by recent developments resulting from attempts to reconcile the proposed function of CFTR with phenomena that are known to be involved in the pathogenesis of the disease. Before the identification of the CFTR gene, many laboratories had described alterations in the glycosylation of CF glycoproteins, but no connection between the altered glycosylation and the pathogenesis of CF was established (for review [2]). This renewed interest fortuitously coincides with the development of automated methods for analysis of the extremely small amounts of relevant oligosaccharides in biologic systems [3].

For the past decade, reports have described CFTR as existing in three different forms, depending on glycosylation: nonglycosylated; core glycosylated; and complex glycosylated, fully mature. It has been reported that only the fully mature form is trafficked to the surface membrane, where it functions as a chloride channel. In some of those studies [4–7] the data have not been completely convincing, although the results have been widely accepted. Nevertheless, the most common mutation ΔF508 has been labeled a processing mutation. It has been reported that, at 26°C, ΔF508 CFTR travels to the surface, where it has chloride channel activity [8]. ΔF508 CFTR is also active when reconstituted into a lipid bilayer [9]. Indeed endogenous ΔF508 CFTR has been identified in the surface membranes of CF cells in culture [10–15]. As yet, no one has directly investigated the carbohydrate structure of CFTR, although many

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; PAGE = polyacrylamide gel electrophoresis; wt = wild-type.

reports have appeared regarding a glycosylation phenotype of material from CF sources (for review [2]). The CF glycosylation phenotype, which is modulated by CFTR, is expressed as decreased sialic acid and an increased amount of fucose linked α1,3 to *N*-acetyl glucosamine [16].

Oligosaccharides of cystic fibrosis transmembrane conductance regulator

Recently, O'Riordan *et al* [17] addressed glycosylation of CFTR directly. Following their earlier studies [18] on the isolation of CFTR from over-expressing Chinese hamster ovary and insect cells, CFTR was isolated from several cell types that were transfected with wtCFTR and that over-expressed it. The over-expression provided those investigators with sufficient CFTR to analyze the oligosaccharide residues in more detail. After immune precipitation the oligosaccharides were released from CFTR with *N*-glycanase and/or endo-β-galactosidase, and were further separated or examined using polyacrylamide gel electrophoresis (PAGE) and lectin (*Datura stramonium* agglutinin and *Maackia amurensis* agglutinin) affinity overlay. *N*-glycanase releases *N*-linked oligosaccharides from the protein, whereas endo-β-galactosidase cleaves polylactosamine containing Galβ1,4GlcNAcβ1,3 repeating units. They also used fluorophore-assisted carbohydrate electrophoresis analysis, a highly sensitive method that provides structural information on purified oligosaccharides.

Interestingly, those investigators found that fully mature, immunopurified CFTR from Chinese hamster ovary cells and a mammary tumor cell line (C127) transfected with wtCFTR contained polylactosamine. T-84 cells (a human colon carcinoma cell line) that were not transfected also had polylactosamine-containing CFTR. The significance of polylactosamine/CFTR would be much greater if endogenous CFTR were extracted from primary human tissue. Unfortunately, the three cell lines have transformed or tumor properties, and polylactosamine is known to occur in tumor cells [19] and Chinese hamster ovary cells [20]. Perhaps this is precisely why the authors did not expand on a relationship to the three size classes of CFTR. In addition, transformed and tumor cells also have an abundance of tri-antennary and tetra-antennary oligosaccharides, so this may have influenced their results [21]. A case in point is the analysis of CFTR from insect cells, Sf9, which were infected with wtCFTR/baculovirus. In this case the authors reported that CFTR contained the insect glycosylation phenotype and was not fully glycosylated. As the authors pointed out, the glycosylation phenotype is influenced by the host's enzymes; however, C127 cells transfected with ΔF508 CFTR showed the PAGE position of core glycosylated CFTR. If confirmed that CFTR isolated from C127 cells after transfection with ΔF508 was core glycosylated, then one has to assume that factors in addition to the host enzymes influence glycosylation of transfected cells. However, this mutant CFTR was not analyzed.

ΔF508 cystic fibrosis transmembrane conductance regulator

O'Riordan *et al* [17] pointed out that the elongation of polylactosamine, in the Golgi at 21°C [22], correlates with the fact that ΔF508 will traffic to the membrane and have chloride channel activity at a reduced temperature [8]. They suggest that, at lower temperature, ΔF508 arrives at and trafficks through the Golgi at a slow rate and is polylactosaminylated. Another possibility is that an existing polylactosamine is elongated, and the glycosylation pattern of ΔF508 CFTR must be examined further to differentiate between these mechanisms. In recent studies, which are yet to be confirmed, a tissue-specific variation of ΔF508 CFTR expression from null to apparently normal amounts indicated that ΔF508 CFTR maturation can be modulated, suggesting that determinants other than CFTR mislocalization may play a role in ΔF508 CF respiratory and intestinal disease [23]. Trafficking between the endoplasmic reticulum and the Golgi appears more complex than was originally believed, and it has recently been proposed that wtCFTR follows a unique pathway [24].

In investigations into CFTR in immortalized airway epithelial cells, Wei *et al* [10] compared morphologically identifiable surface membranes with total cell membrane preparations containing intracellular membranes. Surface membrane CFTR had lower turnover defined by pulse/chase ratios than that of the total cell membrane preparations. Moreover, in the presence of 50 μmol/l castanospermine, an inhibitor of processing α-glucosidases that prevents binding to calnexin, a more rapid turnover of mutant CFTR was found in the total cell membrane preparation, whereas wtCFTR had a slower response. The results are compatible with a pool of CFTR in or near the surface membranes that has an altered turnover in CF and a glycosylation-dependent alteration in the processing of mutant CFTR [10]. It will be interesting to determine whether the surface membrane population of CFTR molecules is for use exclusively as channel proteins, whereas within the cell more than one function may be attributed to the protein.

A hypothesis has been proposed [25] that wtCFTR, by virtue of its proton pump function, contributes to Golgi processing and sorting [26] in airway epithelial cells. The hypothesis is supported by recent reports that the relevant activities of glycosyltransferases and levels of mRNA are the same in both CF and non-CF airway cells, despite the differences in the amounts of fucose and sialic acid in terminal positions in their cell surface membrane glycoproteins [16,25]. ΔF508 CFTR is proposed to affect glycoprotein processing in the Golgi, causing faulty compartmentalization of the glycosyltransferases, which results in the CF glycosylation phenotype. That is, if α1,3-fucosyltransferase is sorted to a compartment positioned prior to α1,2-fucosyltransferase and sialyl transferase, then the activity of the latter two enzymes would be

decreased because the same substrate is required for all three enzymes [27]. The glycosylation pattern of wt and mutant CFTR could influence these events.

It has been shown with the use of over-expressing mutants that the features that determine the processing of CFTR are distinct from those that determine channel function [28]. Cunningham *et al* [29] postulated that the regulatory sites for CFTR trafficking must be either at the trans-Golgi network or peripheral vesicular pools, because the movement of CFTR out of the trans-Golgi network appears to control the onset of CFTR-mediated chloride secretion in a Brefeldin-sensitive pathway [30]. It has been proposed that a difference between CF and non-CF is the inability of ΔF508 CFTR to break out of a recycling pathway between surface membrane and a closely linked compartment [10]. Prince *et al* [31] reported a lack of internalization of wtCFTR with the addition of cAMP. That hyposialylation occurs in CF (for review [2,32]) may suggest that mutant CFTR does not return as efficiently to the trans-Golgi in its recycling pathway. The lack of proper oligosaccharides on the protein may cause this stable form of CFTR to be more sensitive to proteases, and hence to be degraded. It is also possible that both function and localization could independently vary in different cell types and different organs, accounting for the multiple phenotypes that characterize CF.

Conclusion

The methods utilized by O'Riordan *et al* [17] have given the most complete information on the glycosylation of CFTR to date. Those investigators should be congratulated and encouraged to pursue these studies with endogenous human CFTR. The micromethods for oligosaccharide analysis are in place, and both CF and non-CF airway tissues are available. When these analyses are complete, earlier studies can be reassessed and this will provide a more cohesive picture of the relationship of CFTR to glycosylation and of how CFTR is affected by glycosylation.

The key roles of oligosaccharide structures in some disease processes have recently been emphasized [3,33]. As pointed out by O'Riordan *et al* [17], the glycosylation of CFTR has potential implications for the treatment of CF. The determination the oligosaccharide structures of both mutant and wtCFTR from primary human tissue should provide new insights into the pathogenesis of CF. These new insights will provide the foundation for the development of new therapies for CF.

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