Themed Issue: Drug Addiction - From Basic Research to Therapies

Guest Editors - Rao Rapaka and Wolfgang Sadée

Mutant Glycosyltransferases Assist in the Development of a Targeted Drug Delivery System and Contrast Agents for MRI

Submitted: December 22, 2005; Accepted: February 6, 2006; Published: March 24, 2006

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ABSTRACT

The availability of structural information on glycosyltransferases is beginning to make structure-based reengineering of these enzymes possible. Mutant glycosyltransferases have been generated that can transfer a sugar residue with a chemically reactive unique functional group to a sugar moiety of glycoproteins, glycolipids, and proteoglycans (glycoconjugates). The presence of modified sugar moiety on a glycoprotein makes it possible to link bioactive molecules via modified glycan chains, thereby assisting in the assembly of bionanoparticles that are useful for developing the targeted drug delivery system and contrast agents for magnetic resonance imaging. The reengineered recombinant glycosyltransferases also make it possible to (1) remodel the oligosaccharide chains of glycoprotein drugs, and (2) synthesize oligosaccharides for vaccine development.

KEYWORDS: mutant glycosyltransferases, linkages via glycans, glycotargeting, drug delivery systems, cross-linking MRI agents via glycans

INTRODUCTION

Because of the many physiological barriers that must be overcome, targeting drugs to the proposed site of action is a challenging task. The passage of a drug across the endothelium is dependent upon several factors, including its molecular weight and size. Small molecules are taken up by a cell: a process that generally involves a specific transport mechanism or a passive diffusion process. In contrast, uptake of macromolecules by a cell involves receptor-mediated endocytosis or pinocytosis.¹⁻³ These processes involve the interactions between the ligand and the receptors.^{4,5}

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Since oligosaccharides are attached at unique sites in proteins and glycolipids, they bring about specific geometries of interactions between the 2 glycoconjugates, resulting in the correct and efficient molecular interplay between various molecular structures. Owing to their conformational flexibility, the torsional angles of a disaccharide unit, particularly around the 1-6-linkage, adjust in a way that causes the side groups of the oligosaccharides to orient themselves in a manner that produces specific protein-carbohydrate or protein-protein interactions.⁶ Branched oligosaccharides cross-link proteins and generate homo- and heterodimerization of glycoproteins and infinite networks of protein-carbohydrate complexes,⁷⁻⁹ resulting in the modulation of various cellular responses.

Oligosaccharides participate in a large number of biological events, such as cell-cell recognition and adhesion,¹⁰⁻¹³ host-pathogen interactions,¹⁴⁻¹⁶ innate and acquired immunity,^{17,18} and the recruitment of neutrophils to sites of tissue damage.¹⁹ Together with chaperones, they play an important role in the *in vivo* folding and transport of glycoproteins across cellular compartments.²⁰⁻²² They are also the recognition molecules for lectins, the multivalent carbohydrate binding proteins widely found in nature.²³ In animals, a family of β -galactoside binding proteins known as galectins perform several intracellular activities, namely, cell cycle control, anti-apoptosis, and RNA processing.^{24,25} Also, glycosaminoglycans, the carbohydrate moieties of proteoglycans, take part in a wide range of biological functions.²⁶ For example, glycosaminoglycans are involved in the formation of an extracellular matrix to which growth factors bind with a high degree of specificity and, thereby, regulate growth factor activity.²⁷⁻³⁰

Because oligosaccharide moieties of glycoconjugates are involved in many biological processes, they have been used as a tool for the delivery of ligands to specific sites and tissues. The discovery of the hepatic receptor, the asialoglycoprotein receptor (ASGPR), which binds to and internalizes the circulating glycoproteins or glycopeptides that have galactose or *N*-acetylgalactosamine residue at the nonreducing terminus of the oligosaccharide chain,³¹ facilitated “glycotargeting,” the use of carbohydrate ligands to target protein receptors at the site of location.³²⁻³⁵ Here we describe

a method of modifying an oligosaccharide chain of a glycoprotein with mutant glycosyltransferases that allows the linking of 2 glycoconjugates via glycan chains. This strategy can be used for developing a targeted drug delivery system and contrast agents for MRI.

OLIGOSACCHARIDE BIOSYNTHESIS AND GLYCOSYLTRANSFERASES

The biosynthesis of oligosaccharide chains of glycoconjugates involves intricate machinery in a cell, where, from a small number of saccharide units, diverse oligosaccharide structures are generated through isomeric linkages between sugars, each linkage catalyzed by a different enzyme.³⁶ This large family of enzymes, called glycosyltransferases, is involved in the assembly of oligosaccharide chains, each enzyme transferring a monosaccharide moiety of an activated sugar donor to an oligosaccharide acceptor molecule. These enzymes often require a metal ion cofactor. They are grouped into families based on the type of sugar they transfer (eg, galactosyltransferase,³⁷⁻³⁹ fucosyl transferase,⁴⁰ sialyltransferase,⁴¹ polypeptide-N-acetylgalactosaminyltransferase,⁴² O-xylosyltransferase,⁴³ blood group A and B transferases⁴⁴), and within the family there are subfamilies and members. Each transferase generates a specific linkage with a specific acceptor. These enzymes reside in the secretory pathway, although some have also been shown to be present at the cell surface interacting with the cell matrix.^{45,46} They have been implicated as signaling molecules for pattern formation during development.⁴⁷⁻⁴⁹ Chemical structures of many carbohydrate moieties change during cellular development, differentiation, and tumorigenesis.⁵⁰⁻⁵² The synthesis of these carbohydrate moieties depends on the expression of corresponding glycosyltransferases in the cell. Alterations in glycosyltransferase activities that result in the defective glycan synthesis have been shown to have serious pathological consequences leading to several human diseases.⁵³

GENERAL STRUCTURAL FEATURES OF GLYCOSYLTRANSFERASES

Recent structural analysis of the catalytic domain of several glycosyltransferases have revealed that these enzymes are globular proteins that have 2 types of fold, termed GT-A and GT-B, each having an N-terminal and a C-terminal domain.^{54,55} The enzymes of the GT-A fold have 2 dissimilar domains. Mammalian galactosyltransferases (Gal-T), N-acetylglucosaminyltransferases (Gln-T), glucuronyltransferases (GlcA-T), and blood groups A and B transferases have GT-A fold. The N-terminal domain, which recognizes the sugar-nucleotide donor, comprises several β -strands that are each flanked by α -helices, as in a Rossmann-like fold, whereas the C-terminal domain, which contains the acceptor binding site, consists largely of mixed

β -sheets. By contrast, enzymes with the GT-B fold contain 2 similar Rossmann-like folds, with the N-terminal domain providing the acceptor binding site and the C-terminal domain providing the donor binding site. Fungal and phage glucosyltransferases belong to this fold. In both types of enzyme, the 2 domains are connected by a linker region, and the active site is located between the 2 domains. A metal binding site is also located in the cleft in enzymes of both the GT-B and GT-A fold.

The structural work performed on the β -1,4-galactosyltransferase-1 (β 4Gal-T1) in our laboratory,⁵⁶ and on other glycosyltransferases by various other laboratories,⁵⁵ has shown that, upon binding the sugar-nucleotide donor substrate, flexible loops at the substrate binding site of these enzymes undergo a marked conformational change, from an open to a closed conformation⁵⁵ (Figure 1). This change creates an oligosaccharide acceptor binding site in the enzyme that did not exist before. The loop then acts as a lid covering the bound donor substrate. After the transfer of the glycosyl unit to the acceptor, the saccharide product is ejected, and the loop reverts to its native conformation to

Figure 1. Molecular structure of β 4Gal-T1 in closed conformation, in complex with UDP-Gal and Mn^{2+} . The long flexible loop (orange loop, residues Ile345-His365) and the small flexible loop (yellow, residues Gly313-Gly316) are in closed conformation. The Trp314 (W314) side chain is facing toward the binding pocket that is interacting with UDP-Gal. The 3 protein ligands, Asp (D254), Met (M344), and His (H347), the 2 phosphate oxygens (red balls), and 1 molecule of water (yellow ball) participate in the coordination of Mn^{2+} .⁵⁶ The acceptor binding site (shown with arrow) involves 2 Tyr residues, Y289 and Y286, and Phe (F360). The enzyme can be considered as a 2-domain protein, one α -helix rich and the other β -sheet rich, with substrates binding between the domains.

release the remaining nucleotide moiety. This conformational change in β 4Gal-T1 also creates the binding site for α -lactalbumin, a protein produced in the mammary glands during lactation. The interaction of α -lactalbumin with β 4Gal-T1 changes the acceptor specificity of the enzyme from *N*-acetylglucosamine (GlcNAc) to glucose (Glc), which produces lactose that is secreted in milk.

A FEW PROTEIN RESIDUES IN ACTIVE SITE DETERMINE DONOR SPECIFICITY OF GLYCOSYLTRANSFERASES

The specificity of the sugar donor is determined by a few residues in the sugar-nucleotide binding pocket of the enzyme, which are conserved among the family members from different species⁵⁵ (Figure 1). For example, a highly conserved tyrosine residue in β 4Gal-T subfamily members (Tyr289 in bovine β 4Gal-T) determines enzyme specificity toward the galactose moiety of the sugar donor (Figure 2).⁵⁷ By forming a hydrogen bond with its *N*-acetyl group, it restricts the transfer of GalNAc moiety from uridine 5'-diphosphate (UDP)-GalNAc to GlcNAc (Figure 2A). Mutation of Tyr289 to leucine, isoleucine, or asparagine, however, makes the enzyme as efficient as a galactose- or GalNAc-transferase (Figure 2B).⁵⁷ In GlcA-T1, the Ne2 nitrogen of His308 (His311 in GlcAT-P) forms a hydrogen bond with the 2-hydroxyl of GlcUA,^{58,59} which determines enzyme specificity toward UDP-GlcUA as the sugar donor. Substi-

tution of His308 with arginine in GlcAT-I enables the enzyme to efficiently use UDP-Glc, UDP-Man, or UDP-GlcNAc as the donor substrates.⁶⁰ Met266 and Ala268 in the blood group B Gal-T, and Leu266 and Gly268 in the blood group A GalNAc transferase, determine specificity toward their respective sugar donors, UDP-Gal and UDP-GalNAc.⁶¹ Spontaneous mutation of a residue that confers donor specificity to a residue that broadens it, such as the mutation of tyrosine to isoleucine in β 4Gal-T1 or histidine to arginine in GlcAT-I, will result in cellular glycans with altered sugar residues and could have significant effects on normal cellular processes.

MUTATIONS OF DONOR BINDING RESIDUES BROADEN DONOR SPECIFICITY OF THE ENZYME: MUTANT ENZYME Y289L-GAL-T1 TRANSFERS 2-KETO-GALACTOSE TO GLCNAc RESIDUE

Based on the structural information, mutant enzymes have been generated that have broader donor-sugar specificity.^{57,62} Tyr289 residue in bovine β 4Gal-T 1, and Tyr or Phe residue at the corresponding positions in other β 4Gal-T family members of various species determine the specificity toward the donor sugar nucleotide UDP-Gal. Mutation of Tyr289 to Leu or Ile both abrogates the hydrogen-bonding capacity as well as enlarges the binding pocket such that the mutant enzyme Y289L-Gal-T transfers GalNAc from the donor substrate UDP-GalNAc with as good efficiency as

Figure 2. (A) Crystal structure of the wild-type β 4Gal-T1 in complex with UDP-GalNAc shows⁵⁷ that Tyr 289 makes hydrogen bond interactions with the *N*-acetyl-group of GalNAc residue (arrow), thereby hindering its transfer to the acceptor residue GlcNAc (0.1% activity). (B) This hindrance is removed when Tyr 289 is mutated to Leu 289. The mutant enzyme, Y289L-Gal-T1, transfers GalNAc⁵⁷ or 2-modified galactose⁶³ from the corresponding UDP-derivatives to GlcNAc as efficiently as the wild type transfers Gal from UDP-Gal to GlcNAc.

Gal from UDP-Gal (Figure 2B). The mutant enzyme Y289L-Gal-T can also transfer from the UDP derivative the galactose moiety that has substitutions other than the 2-*N*-acetyl group (eg, $-\text{CH}_2\text{-CO-CH}_3$, located at the C-2 position⁶³) (Figure 3A). The ketone moiety of the substituting group is a versatile chemical handle. Under mild conditions (pH 6.7 buffer, 25°C), it can be coupled to an aminooxy biotin derivative, *N*-(aminooxyacetyl)-*N'*-(D-biotinoyl) hydrazine (Figure 3B). The biotinylated product can then be detected with streptavidin-horse radish peroxidase (HRP).⁶³

COUPLING OF GLYCOPROTEINS VIA OLIGOSACCHARIDE CHAINS AND THE GENERAL UTILITY OF THE TECHNIQUE

The availability of mutant glycosyltransferases makes it possible to transfer sugar moieties with a variety of functional groups from their respective donor nucleotides^{63,64} to a specific residue of an oligosaccharide chain of a glycoprotein. The 2 glycoproteins, each carrying the sugar moiety with reactive functional groups, can then be coupled by heterobifunctional linkers (Figure 4), thus enabling 2 glycoproteins to be coupled at a specific site. One glycoprotein can be a monoclonal antibody, which carries a single *N*-linked oligosaccharide chain on the immunoglobulin G (IgG) heavy chain, while another can be a toxin. This coupling

Figure 4. General scheme for generating modified glycans of glycoproteins with mutant glycosyltransferases, which can be used for the cross-linking and molecular assembly of 2 glycoproteins, A and B. A modified galactose residue, X or Y, is transferred from its respective UDP derivative, by the mutant enzyme to *N*-acetylglucosamine residue at the nonreducing terminus of the oligosaccharide chain of each glycoprotein, A or B. The X and Y functional groups on galactose residues are such that they can be linked by a heterobifunctional linker; each functional group reacts only with the X or Y group of the galactose residue, respectively.

allows a controlled and specific linking of monoclonal antibodies to toxins. Furthermore, the monoclonal antibodies whose *N*-linked glycan moiety has been modified to have 2-keto-galactose can be linked to aminooxy linkers carrying chelating agents specific for gadolinium or other contrast agents for imaging.

In the currently prevailing methods, generally 2 proteins are cross-linked, using a bifunctional cross-linker, at random sites to a protein residue (eg, lysine), which is distributed at several places on the protein surface. This method of cross-linking often blocks the functional sites on the protein and thus reduces the bioefficacy of the protein. In contrast, the advantage of the method described here, in which 2 glycoproteins or glycoconjugates are linked via glycan chains, is that the linkage between the 2 partners is at a defined site, where glycan moiety is attached to the glycoconjugates. For example, a cargo can be attached to a monoclonal antibody at the asparagine-linked glycan chain, which resides in the Fc-fragment, away from the antigen binding site, and delivered to the site of action without altering the bioefficacy of the monoclonal antibody.

CONCLUSIONS

The structural information of glycosyltransferases has made it possible to design novel glycosyltransferases with broader and requisite donor specificities. The specificity of the donor sugar of these enzymes is determined by a few residues in their sugar-nucleotide binding pocket, which are conserved among the family members from different species. Based

Figure 3. (A) Schematics of the transfer of a modified sugar by the mutant β -1,4-galactosyl-transferase, Y289L-Gal-T1, to *N*-acetylglucosamine moiety of a glycoconjugate. (B) Coupling of the modified sugar residue with an aminooxy-linker.

on the structural information and conformational analysis of oligosaccharide chains, we have been able to design novel glycosyltransferases with broader donor and acceptor specificities. Several mutant glycosyltransferases have been generated that can transfer a sugar residue with a chemically reactive functional group to N-acetylglucosamine residue of a glycoconjugate. The modified oligosaccharide moieties of glycoconjugates can then be used for linking bioactive molecules via glycan chains, thereby assisting in the assembly of bionanoparticles that are useful for developing the targeted drug delivery system and contrast agents for MRI.

ACKNOWLEDGMENTS

This research was supported by the Intramural Research Program of the National Institutes of Health (NIH), National Cancer Institute (NCI), Center for Cancer Research, Frederick, MD. The content of this publication does not necessarily reflect the view or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government. This project has been funded in part with federal funds from the NCI, NIH, under contract No. N01-C0-12400.

REFERENCES

1. Mukherjee S, Ghosh RN, Maxfield FR. Endocytosis. *Physiol Rev*. 1997;77:759-803.
2. Gao H, Shi W, Freund LB. Mechanics of receptor-mediated endocytosis. *Proc Natl Acad Sci USA*. 2005;102:9469-9474.
3. Bao G, Bao XR. Shedding light on the dynamics of endocytosis and viral budding. *Proc Natl Acad Sci USA*. 2005;102:9997-9998.
4. Vyas SP, Singh A, Sihorkar V. Ligand-receptor-mediated drug delivery: an emerging paradigm in cellular drug targeting. *Crit Rev Ther Drug Carrier Syst*. 2001;18:1-76.
5. Russell-Jones GJ. The potential use of receptor-mediated endocytosis for oral drug delivery. *Adv Drug Deliv Rev*. 2001;46:59-73.
6. Qasba PK. Involvement of sugars in protein-protein interactions. *Carbohydrate Polymers*. 2000;41:293-309.
7. Bourne Y, Bolgiano B, Liao DI, et al. Crosslinking of mammalian lectin (galectin-1) by complex bi-antennary saccharides. *Nat Struct Biol*. 1994;1:863-870.
8. Bourne Y, Roussel A, Frey M, Rouge P, Fontecilla-Camps JC, Cambillau C. Three-dimensional structures of complexes of Lathyrus ochrus isolectin I with glucose and mannose: fine specificity of the monosaccharide-binding site. *Proteins*. 1990;8:365-376.
9. Wright CS. Crystal structure of a wheat germ agglutinin/glycophorin-sialoglycopeptide receptor complex: structural basis for cooperative lectin-cell binding. *J Biol Chem*. 1992;267:14345-14352.
10. Crocker PR, Feizi T. Carbohydrate recognition systems: functional triads in cell-cell interactions. *Curr Opin Struct Biol*. 1996;6:679-691.
11. Collins BE, Paulson JC. Cell surface biology mediated by low affinity multivalent protein-glycan interactions. *Curr Opin Chem Biol*. 2004;8:617-625.

12. Akama TO, Nakagawa H, Sugihara K, et al. Germ cell survival through carbohydrate-mediated interaction with Sertoli cells. *Science*. 2002;295:124-127.
13. Talbot P, Shur BD, Myles DG. Cell adhesion and fertilization: steps in oocyte transport, sperm-zona pellucida interactions, and sperm-egg fusion. *Biol Reprod*. 2003;68:1-9.
14. Sharon N, Ofek I. Fighting infectious diseases with inhibitors of microbial adhesion to host tissues. *Crit Rev Food Sci Nutr*. 2002;42:267-272.
15. Sharon N. Carbohydrate-lectin interactions in infectious disease. *Adv Exp Med Biol*. 1996;408:1-8.
16. Sacks D, Kamhawi S. Molecular aspects of parasite-vector and vector-host interactions in leishmaniasis. *Annu Rev Microbiol*. 2001;55:453-483.
17. Rudd PM, Wormald MR, Stanfield RL, et al. Roles of glycosylation of cell surface receptors involved in cellular immune recognition. *J Mol Biol*. 1999;293:351-366.
18. Rudd PM, Elliott T, Cresswell P, Wilson IA, Dwek RA. Glycosylation and the immune system. *Science*. 2001;291:2370-2376.
19. Lowe JB. Glycan-dependent leukocyte adhesion and recruitment in inflammation. *Curr Opin Cell Biol*. 2003;15:531-538.
20. Parodi AJ. Role of N-oligosaccharide endoplasmic reticulum processing reactions in glycoprotein folding and degradation. *Biochem J*. 2000;348:1-13.
21. Trombetta ES, Helenius A. Lectins as chaperones in glycoprotein folding. *Curr Opin Struct Biol*. 1998;8:587-592.
22. Ghosh P, Dahms NM, Kornfeld S. Mannose 6-phosphate receptors: new twists in the tale. *Nat Rev Mol Cell Biol*. 2003;4:202-212.
23. Sharon N, Lis H. History of lectins: from hemagglutinins to biological recognition molecules. *Glycobiology*. 2004;14:53R-62R.
24. Yang RY, Liu FT. Galectins in cell growth and apoptosis. *Cell Mol Life Sci*. 2003;60:267-276.
25. Liu F, Patterson RJ, Wang JL. Intracellular functions of galectins. *Biochim Biophys Acta*. 2002;1572:263-273.
26. Handel TM, Johnson Z, Crown SE, Lau EK, Sweeney M, Proudfoot AE. Regulation of protein function by glycosaminoglycans - as exemplified by chemokines. *Annu Rev Biochem*. 2005;74:385-410.
27. Schlessinger J, Lax I, Lemmon M. Regulation of growth factor activation by proteoglycans: what is the role of the low affinity receptors? *Cell*. 1995;83:357-360.
28. Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM. Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. *Cell*. 1991;64:841-848.
29. Herr AB, Ornitz DM, Sasisekharan R, Venkataraman G, Waksman G. Heparin-induced self-association of fibroblast growth factor-2: evidence for 2 oligomerization processes. *J Biol Chem*. 1997;272:16382-16389.
30. Lam K, Rao VSR, Qasba PK. Molecular modeling studies on binding of bFGF to heparin and its receptor FGFR1. *J Biomol Struct Dyn*. 1998;15:1009-1027.
31. Morell AG, Gregoriadis G, Scheinberg H, Hickman J, Ashwell G. The role of sialic acid in determining the survival of glycoproteins in the circulation. *J Biol Chem*. 1971;246:1461-1467.
32. Rogers JC, Kornfeld S. Hepatic uptake of proteins coupled to fetuin glycopeptide. *Biochem Biophys Res Commun*. 1971;45:622-629.
33. Lee YC, Townsend RR, Hardy MR, et al. Binding of synthetic oligosaccharides to the hepatic Gal/GalNAc lectin: dependence on fine structural features. *J Biol Chem*. 1983;258:199-202.

34. Hangeland JJ, Levis JT, Lee YC, Tso POP. Cell-type specific and ligand specific enhancement of cellular uptake of oligodeoxynucleoside methylphosphonates covalently linked with a neoglycopeptide, YEE(ah-GalNAc). *Bioconj Chem*. 1995;6:695-701.
35. Weigel PH, Yik JH. Glycans as endocytosis signals: the cases of the asialoglycoprotein and hyaluronan/chondroitin sulfate receptors. *Biochim Biophys Acta*. 2002;1572:341-363.
36. Roseman S. Reflections on glycobiology. *J Biol Chem*. 2001;276:41527-41542.
37. Amado M, Almeida R, Schwientek T, Clausen H. Identification and characterization of large galactosyltransferase gene families: galactosyltransferases for all functions. *Biochim Biophys Acta*. 1999;1473:35-53.
38. Hennot T. The galactosyltransferase family. *Cell Mol Life Sci*. 2002;59:1081-1095.
39. Berger EG, Rohrer J. Galactosyltransferase: still up and running. *Biochimie*. 2003;85:261-274.
40. Javaud C, Dupuy F, Maftah A, Julien R, Petit JM. The fucosyltransferase gene family: an amazing summary of the underlying mechanisms of gene evolution. *Genetica*. 2003;118:157-170.
41. Harduin-Lepers A, Vallejo-Ruiz V, Krzewinski-Recchi MA, Samyn-Petit B, Julien S, Delannoy P. The human sialyltransferase family. *Biochimie*. 2001;83:727-737.
42. Ten Hagen KG, Fritz TA, Tabak LA. All in the family: the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases. *Glycobiology*. 2003;13:1R-16R.
43. Wilson IB. The never-ending story of peptide O-xylosyltransferase. *Cell Mol Life Sci*. 2004;61:794-809.
44. Palcic MM, Seto NO, Hindsgaul O. Natural and recombinant A and B gene encoded glycosyltransferases. *Transfus Med*. 2001;11:315-323.
45. Shur BD, Evans S, Lu Q. Cell surface galactosyltransferase: current issues. *Glycoconj J*. 1998;15:537-548.
46. Rodeheffer C, Shur BD. Targeted mutations in β 1,4-galactosyltransferase I reveal its multiple cellular functions. *Biochim Biophys Acta*. 2002;1573:258-270.
47. Haltiwanger RS, Stanley P. Modulation of receptor signaling by glycosylation: fringe is an O-fucose-beta,3-N-acetylglucosaminyltransferase. *Biochim Biophys Acta*. 2002;1573:328-335.
48. Nakamura Y, Haines N, Chen J, et al. Identification of a Drosophila gene encoding xylosylprotein beta4-galactosyltransferase that is essential for the synthesis of glycosaminoglycans and for morphogenesis. *J Biol Chem*. 2002;277:46280-46288.
49. Takahashi M, Tsuda T, Ikeda Y, Honke K, Taniguchi N. Role of N-glycans in growth factor signaling. *Glycoconj J*. 2004;20:207-212.
50. Feizi T. Demonstration by monoclonal antibodies that carbohydrate structures of glycoproteins and glycolipids are onco-developmental antigens. *Nature*. 1985;314:53-57.
51. Hakomori S. Glycosylation defining cancer malignancy: new wine in an old bottle. *Proc Natl Acad Sci USA*. 2002;99:10231-10233.
52. Couldrey C, Green JE. Metastases: the glycan connection. *Breast Cancer Res*. 2000;2:321-323.
53. Freeze HH, Aebi M. Altered glycan structures: the molecular basis of congenital disorders of glycosylation. *Curr Opin Struct Biol*. 2005;15:490-498.
54. Bourne Y, Henrissat B. Glycoside hydrolases and glycosyltransferases: families and functional modules. *Curr Opin Struct Biol*. 2001;11:593-600.
55. Qasba PK, Ramakrishnan B, Boeggeman E. Substrate-induced conformational changes in glycosyltransferases. *Trends Biochem Sci*. 2005;30:53-62.
56. Ramakrishnan B, Boeggeman E, Ramasamy V, Qasba PK. Structure and catalytic cycle beta-1,4-galactosyltransferase. *Curr Opin Struct Biol*. 2004;14:593-600.
57. Ramakrishnan B, Qasba PK. Structure-based design of beta-1,4-galactosyl-transferase-I (beta 4Gal-T1) with equally efficient N-acetylgalactosaminyltransferase activity. *J Biol Chem*. 2002;277:20833-20840.
58. Pedersen LC, Darden TA, Negishi M. Crystal structure of beta 1,3-glucuronyltransferase I in complex with active donor substrate UDP-GlcUA. *J Biol Chem*. 2002;277:21869-21873.
59. Kakuda S, Shiba T, Ishiguro M, et al. Structural basis for acceptor substrate recognition of a human glucuronyltransferase, GlcAT-P, an enzyme critical in the biosynthesis of the carbohydrate epitope HNK-1. *J Biol Chem*. 2004;279:22693-22703.
60. Ouzzine M, Gulberti S, Levoine N, Netter P, Magdalou J, Fournel-Gigleux S. The donor substrate specificity of the human beta 1,3-glucuronosyltransferase I toward UDP-glucuronic acid is determined by two crucial histidine and arginine residues. *J Biol Chem*. 2002;277:25439-25445.
61. Marcus SL, Polakowski R, Seto NO, et al. A single point mutation reverses the donor specificity of human blood group B-synthesizing galactosyltransferase. *J Biol Chem*. 2003;278:12403-12405.
62. Ramakrishnan B, Boeggeman E, Qasba PK. Mutation of Arg 228 to lysine enhances the glucosyltransferase activity of bovine beta-1,4-galactosyltransferase I. *Biochemistry*. 2005;44:3202-3210.
63. Khidekel N, Arndt S, Lamarre-Vincent N, et al. A chemoenzymatic approach toward the rapid and sensitive detection of O-GlcNAc posttranslational modifications. *J Am Chem Soc*. 2003;125:16162-16163.
64. Mizanur RM, Jaipuri FA, Pohl NL. One-step synthesis of labeled sugar nucleotides for protein O-GlcNAc modification studies by chemical function analysis of an archaeal protein. *J Am Chem Soc*. 2005;127:836-837.