Metabolism of *N*-Glycolylneuraminic Acid in Human and Nonhuman Cells, and Potential Relationships to Human Disease

161

Anne K. Bergfeld, Annie N. Samraj, and Ajit Varki

Contents

Abstract

N-Glycolylneuraminic acid (Neu5Gc) is a sialic acid commonly found at the outermost position of glycan chains at the surface of most mammalian cells. The only known pathway for synthesis of Neu5Gc is the conversion of CMP-*N*-acetylneuraminic acid (CMP-Neu5Ac) to CMP-Neu5Gc, catalyzed by the cytidine monophospho-*N*-acetylneuraminic acid hydroxylase (Cmah). These CMP-activated sialic acids serve as precursors for glycan assembly in the Golgi apparatus. A degradative pathway to eliminate Neu5Gc and regulate Neu5Gc levels in mammalian cells involves enzymatic conversion of Neu5Gc to *N*-glycolylglucosamine-6-phosphate (GlcNGc-6P) via *N*-glycolylmannosamine (ManNGc) and GlcNGc. Irreversible de-*N*-glycolylation of GlcNGc-6P generates the common cellular metabolites glucosamine-6-phosphate and glycolate and therewith eliminates the *N*-glycolyl group from sialic acid and amino sugar biosynthetic pathways. Humans lack endogenous Neu5Gc biosynthesis due to an inactivating deletion in the human *CMAH* gene. This has been confirmed by the absence of detectable Neu5Gc in the *Cmah*$^{-/-}$ mouse model harboring the humanlike *Cmah* allele. However, humans incorporate exogenous Neu5Gc from

A.K. Bergfeld • A.N. Samraj • A. Varki (✉)
Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA, USA
e-mail: abergfeld@ucsd.edu; asamraj@ucsd.edu; a1varki@ucsd.edu

N. Taniguchi et al. (eds.), *Glycoscience: Biology and Medicine*,
DOI 10.1007/978-4-431-54841-6_169

animal-derived food products (primarily meats of mammalian origin) into endogenous cellular glycoconjugates despite a polyclonal anti-Neu5Gc antibody response, which makes Neu5Gc the first known "xeno-autoantigen" in humans. The metabolic pathways and potential implications for human disease are discussed.

Keywords

Sialic acid • *N*-Glycolylneuraminic acid (Neu5Gc) • Metabolism of Neu5Gc • *N*-Glycolylglucosamine (GlcNGc) • *N*-Glycolylgalactosamine (GalNGc) • Cmah • Xenosialitis

Introduction

Sialic acids are a family of over 50 naturally occurring acidic monosaccharides (Varki and Schauer 2009). The two prevalent sialic acids in mammals are *N*-glycolylneuraminic acid (Neu5Gc) and its precursor *N*-acetylneuraminic acid (Neu5Ac). They are commonly found at the terminal positions of glycan chains of cell-surface glycoconjugates in mammals. Sialic acids are CMP activated in the nucleus and subsequently transported via the cytosol to the Golgi apparatus, where they are added onto underlying glycan chain acceptors by sialyltransferases. Neu5Gc is biosynthesized by enzymatic hydroxylation of its precursor Neu5Ac catalyzed by the cytidine monophospho-*N*-acetylneuraminic acid hydroxylase (Cmah). The reaction takes place in the cytosol and converts CMP-Neu5Ac into CMP-Neu5Gc (Bergfeld and Varki 2013). The single copy *CMAH* gene was found to be inactivated in humans due to a frameshift mutation caused by an Alu-mediated elimination of an exon (Chou et al. 1998; Irie et al. 1998). This mutation occurred around 3 million years ago and was subsequently fixed in the human species (Hayakawa et al. 2006). The complete absence of endogenous Neu5Gc biosynthesis due to the loss of Cmah function was confirmed in a mouse model harboring the humanlike mutation of the *Cmah* allele (Hedlund et al. 2007). Regardless, the presence of Neu5Gc has been unambiguously demonstrated in various human samples (Malykh et al. 2001; Higashi et al. 1985; Diaz et al. 2009). The consumption of mammal-derived food products such as red meats is the likely source for trace Neu5Gc in humans (Varki et al. 2011; Banda et al. 2012). The loss of Neu5Gc biosynthesis did not seem to impact human metabolic pathways, which utilize exogenous Neu5Gc with no apparent difference compared to other mammalian cells (Tangvoranuntakul et al. 2003; Bardor et al. 2005). The uptake of free sialic acids is predominantly mediated via macropinocytosis and the lysosomal route for recycling of cell-surface glycoconjugates, as sialic acids are unlikely to directly cross the cell membrane. The same mechanism likely explains the presence of low levels of Neu5Gc in human tissues. It has been shown that radiolabeled, glycoprotein-bound Neu5Gc from the diet can be metabolized in mice and rats by detection of radiolabeled CO_2 (Nohle and Schauer 1984). More recently, it has been demonstrated that mice incorporate exogenous Neu5Gc from Neu5Gc-containing

glycoconjugates of the diet into various endogenous tissues, although the exact pathways from the gut to the circulation remain to be uncovered (Banda et al. 2012). By contrast, the immune system has adapted to the human loss of Neu5Gc and recognizes this foreign molecule as an antigen. The incorporation of dietary Neu5Gc despite a polyclonal immune response with circulating anti-Neu5Gc antibodies in all humans makes Neu5Gc the first known "xeno-autoantigen" (Varki et al. 2011).

Metabolism of *N*-Glycolylneuraminic Acid in Mammalian Cells (Fig. 1)

In mammalian cells, sialic acid metabolism was first described for the major human sialic acid Neu5Ac. However, all enzymes involved also tolerate a variety of Neu5Ac derivatives albeit with different substrate specificities. Similar to Neu5Ac biology, pathways involved in Neu5Gc metabolism are summarized in Fig. 1. Mammalian cells can recycle Neu5Gc from cell-surface and extracellular glycoconjugates by macropinocytosis. Vesicular transport results in delivery to a lysosomal environment where sialidases Neu1 and Neu4 cleave Neu5Gc off the glycan chain. The released monomer can be transported to the cytosol by the lysosomal sialin transporter SLC17A5 (Bardor et al. 2005; Altheide et al. 2006). Cytosolic Neu5Gc can be relocated to the nucleus where the CMP-sialic acid synthetase is available to activate sialic acids. Resulting CMP-Neu5Gc is transported back to the cytosol, which is also the compartment where Neu5Gc *de novo* biosynthesis occurs. The precursor molecule CMP-Neu5Ac is converted to CMP-Neu5Gc by action of CMAH. The activated sialic acids serve as substrates for the transporter SLC35A1 in the Golgi membrane (Altheide et al. 2006). In antiport with CMP, CMP-Neu5Gc enters the Golgi lumen, where it subsequently serves as a precursor for sialyltransferases, which add Neu5Gc to the glycan chains (Harduin-Lepers et al. 2001). The resulting Neu5Gc-containing cellular glycoconjugates are transported to the cell surface or excreted.

In order to regulate Neu5Gc levels as needed, mammalian cells should have a pathway in place to break down sialic acids if necessary. Based on the well-studied pathways for turnover of *N*-acetylhexosamines, one such metabolic route has been identified recently. Reversible conversion of cytosolic Neu5Gc to *N*-glycolylmannosamine (ManNGc) has been described to occur (Schauer et al. 1999). The ManNGc can then be epimerized to *N*-glycolylglucosamine (GlcNGc) by action of GlcNAc 2′-epimerase (Ghosh and Roseman 1965). Phosphorylation of GlcNGc to GlcNGc-6P has been confirmed to be catalyzed by GlcNAc kinase (Bergfeld et al. 2012b). The final step involves the irreversible de-*N*-glycolylation reaction resulting in the common metabolites glucosamine-6-phosphate and glycolate, which is catalyzed by GlcNAc-6-P deacetylase (Bergfeld et al. 2012b). Importantly, the activated form of glycolate, glycolyl-CoA, has been repeatedly reported in literature as a potential alternative (Cmah-independent) route for Neu5Gc biosynthesis in humans (for review, see Malykh et al. 2001). However, glycolate was not found to serve as a suitable precursor for Neu5Gc *de novo* biosynthesis

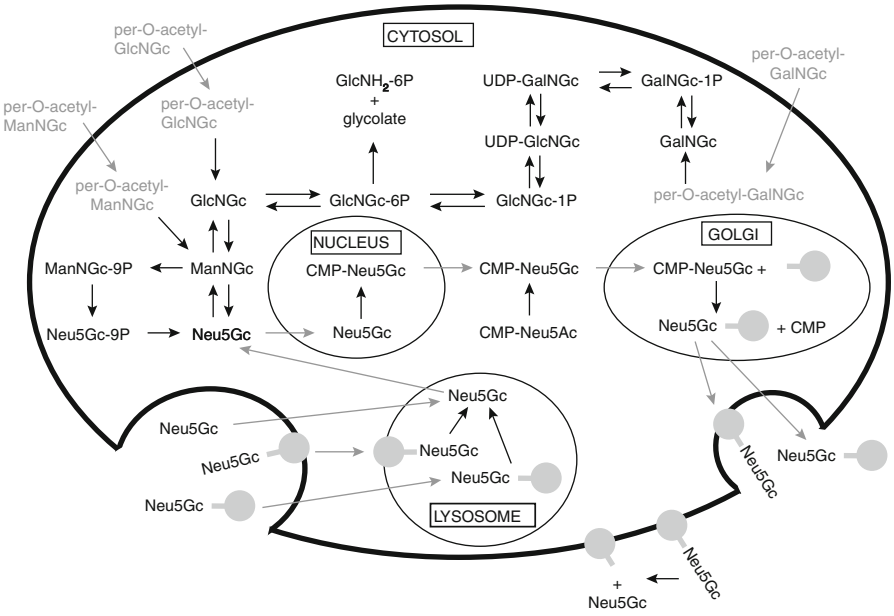

Fig. 1 Schematic representation of Neu5Gc metabolism in a mammalian cell. Glycoconjugate acceptor structures such as glycoproteins and glycolipids are represented as *gray spheres* with the underlying glycan chain as a *gray line*. *Gray font color* is used for artificial monosaccharides that can be used to introduce *N*-glycolyl groups into mammalian cells (Copyright Anne Bergfeld, Annie Samraj, and Ajit Varki)

(Bergfeld et al. 2012b). The absence of Neu5Gc in the $Cmah^{-/-}$ mouse model confirms the necessity of Cmah for Neu5Gc biosynthesis as it harbors the human-like defect in the *Cmah* gene (Hedlund et al. 2007; Diaz et al. 2009).

To modify the cellular pathways for sialic acid biosynthesis, various compounds have been used for supplementing the media of cultured mammalian cell lines. Sialic acid analogues themselves have been synthesized and widely used to determine their affinity to pathway enzymes and the ability to incorporate into cellular glycoconjugates (Feng et al. 2013). As Neu5Ac and its derivatives can only enter the cells via macropinocytosis, compounds that either can use a membrane transporter or are capable of crossing the cell membrane themselves are superior for reaching high intracellular concentrations. A convenient method to significantly enhance uptake of monosaccharides into cells is the peracetylation reaction (Sarkar et al. 1995). For example, cell feedings with per-*O*-acetylated ManNGc increased intracellular Neu5Gc levels up to 100-fold as compared to supplementing cells with equimolar amounts of unmodified ManNGc (Collins et al. 2000). Besides the *N*-glycolyl group, the sialic acid metabolic pathways are very tolerant toward multiple artificial substituents predominantly in the 5-position. For example, *N*-azidoacetylmannosamine (ManNAz) can be tolerated and used to introduce a sugar analogue with a reactive azide function into cellular glycans, which in turn

can be exploited for visualization and capture of glycoconjugates (Saxon and Bertozzi 2000). Media supplementation of cultured cells with synthetic monosaccharides was also used to confirm that mammalian cells incorporate exogenous GlcNGc and can use it for *O*-GlcNGcylation of cellular proteins (Macauley et al. 2012). Moreover, swine cells supplemented with exogenous ManNGc seem to incorporate the *N*-glycolyl group into N-glycans as well as O-glycans (Bateman et al. 2010). Additionally, the GalNAc salvage pathway is a possible route to introduce an *N*-glycolyl group to sialic acid and amino sugar biosynthetic pathways. Supplementation of culturing media of mammalian cells with GalNGc results in incorporation of GalNGc into cellular O-glycans (Pouilly et al. 2011). In parallel, cell feedings with GalNGc were shown to result in biosynthesis of the precursors UDP-GalNGc and UDP-GlcNGc, which were subsequently incorporated into most major glycan classes (Bergfeld et al. 2012a). Moreover, the *N*-glycolyl group introduced via the GalNAc salvage pathway gave rise to biosynthesis of Neu5Gc in Neu5Gc-deficient human cells, confirming once again the remarkable tolerance of mammalian sialic acid metabolic pathways toward substituents at the 5-position (Bergfeld et al. 2012a).

Potential Implications for Human Diseases

The presence of Neu5Gc in human tissues can potentially lead to detrimental consequences either due to Neu5Gc *per se* or due to its interaction with circulating anti-Neu5Gc antibodies. Trace amounts of Neu5Gc have been detected in various human epithelial cancers including breast, colon, lung, prostate, and ovary (Tangvoranuntakul et al. 2003), where it likely aggravates carcinoma risk by the "xenosialitis" phenomenon, i.e., interaction of the xeno-autoantigen Neu5Gc with anti-Neu5Gc antibodies. Chronic inflammation has been proven to be a critical role-player in the progression of cancers, and it is by this mechanism that Neu5Gc-expressing tumors in $Cmah^{-/-}$ mice grow larger in the presence of anti-Neu5Gc antibodies (Hedlund et al. 2008). When the Neu5Gc-containing B16F1 murine melanoma cell line was subcutaneously injected into $Cmah^{-/-}$ mice, anti-Neu5Gc antibodies were induced and tumors grew larger. Similar experiments with MC38 murine cells were associated with larger tumors, enhanced angiogenesis, and leukocyte infiltration. These effects could be curbed by a cyclooxygenase-2 inhibitor or with very high levels of anti-Neu5Gc antibodies. On the other hand, high doses of affinity-purified human anti-Neu5Gc antibodies could suppress tumor growth (Padler-Karavani et al. 2011). These findings can be clarified by our recent proof of Prehn's hypothesis of a dualistic, dose-dependent response to an immunostimulant (Pearce et al. 2014). 14 F7, a monoclonal antibody against (Neu5Gc)GM3 which is a tumor-associated ganglioside seen in skin and breast cancer, is one such example, and trials are currently underway to determine its efficacy (Fernandez-Marrero et al. 2011). Given this experimental evidence, avoidance of Neu5Gc-rich red meat could potentially reduce cancer risk. Neu5Gc is also seen as a contaminant in cancer therapeutic agents such as cetuximab, which results in immune complex formation and faster drug clearance (Ghaderi et al. 2010). Also,

subtilase cytotoxin released by Shiga toxigenic *Escherichia coli* (STEC) has higher binding specificity to α2–3-linked Neu5Gc as compared to α2–3-linked Neu5Ac. Thus, in the absence of Neu5Gc-rich proteins in the serum, circulating toxin likely targets Neu5Gc expressed on endothelial surfaces and other epithelial linings giving rise to hemolytic anemia and hemolytic-uremic syndrome (HUS) (Lofling et al. 2009). *In vitro* experiments prove that Neu5Gc incorporated in human umbilical vein endothelial cells (HUVECs) can interact with anti-Neu5Gc antibodies and lead to antibody-dependent binding, complement deposition, endothelial activation, selectin expression, increased cytokine expression, and monocyte binding. Immunohistochemistry on human hearts and aortas showed accumulation of Neu5Gc in the endothelium overlying atherosclerotic plaques and in the subendothelial regions (Pham et al. 2009). Taken together, this implies that xenosialitis could also play an active role in diseases of vascular inflammation such as atherosclerosis. Elevated levels of anti-Neu5Gc antibodies are seen in Kawasaki disease, a childhood vasculitis. Higher levels are seen in those with normal coronary arteries as opposed to those with complications, i.e., with aneurysms or dilated coronaries, suggesting that it may be used to monitor disease progress (Padler-Karavani et al. 2013). Increased expression of anti-Neu5Gc antibodies from the $Cmah^{-/-}/mdx^{-/-}$ mice results in complement-dependent killing of Neu5Gc loaded primary myoblasts and myotubes suggesting that xenosialitis may even play a role in muscular dystrophy (Chandrasekharan et al. 2010).

Future Directions

A common theme in the diseases described above is chronic inflammation, and evidence thus far appears to implicate the interaction of Neu5Gc with anti-Neu5Gc antibodies as an exacerbating factor. Taken together, it is also tempting to speculate that naturally occurring GlcNGc as an intermediate of the Neu5Gc-degrading pathway may be metabolized to some extent into UDP-GlcNGc and UDP-GalNGc. Such activated monosaccharides could potentially be incorporated into various glycoconjugates in mammalian cells. GlcNGc and GalNGc would thus represent novel naturally occurring monosaccharides, which further increase the variety of mammalian glycan chains. Given that Neu5Gc is a xeno-autoantigen in humans, GlcNGc- or GalNGc-containing glycans would have the same potential of being immunogenic. However, if *N*-glycolylated amino sugars occur naturally in mammalian glycoconjugates, they are likely to be of low abundance; else they would have been described. Further studies are necessary to address these aspects.

References

Altheide TK, Hayakawa T, Mikkelsen TS, Diaz S, Varki N, Varki A (2006) System-wide genomic and biochemical comparisons of sialic acid biology among primates and rodents: evidence for two modes of rapid evolution. J Biol Chem 281:25689–25702

Banda K, Gregg CJ, Chow R, Varki NM, Varki A (2012) Metabolism of vertebrate amino sugars with N-glycolyl groups: mechanisms underlying gastrointestinal incorporation of the non-human sialic acid xeno-autoantigen N-glycolylneuraminic acid. J Biol Chem 287:28852–28864

Bardor M, Nguyen DH, Diaz S, Varki A (2005) Mechanism of uptake and incorporation of the non-human sialic acid N-glycolylneuraminic acid into human cells. J Biol Chem 280:4228–4237

Bateman AC, Karamanska R, Busch MG, Dell A, Olsen CW, Haslam SM (2010) Glycan analysis and influenza A virus infection of primary swine respiratory epithelial cells: the importance of NeuAc{alpha}2-6 glycans. J Biol Chem 285:34016–34026

Bergfeld AK, Varki A (2013) Cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH). In: Taniguchi N, Honke K, Fukuda M, Narimatsu H, Yamaguchi Y, Angata T (eds) Handbook of glycosyltransferases and related genes. Springer, New York

Bergfeld AK, Pearce OM, Diaz SL, Lawrence R, Vocadlo DJ, Choudhury B, Esko JD, Varki A (2012a) Metabolism of vertebrate amino sugars with N-glycolyl groups: incorporation of N-glycolylhexosamines into mammalian glycans by feeding N-glycolylgalactosamine. J Biol Chem 287:28898–28916

Bergfeld AK, Pearce OM, Diaz SL, Pham T, Varki A (2012b) Metabolism of vertebrate amino sugars with N-glycolyl groups: elucidating the intracellular fate of the non-human sialic acid N-glycolylneuraminic acid. J Biol Chem 287:28865–28881

Chandrasekharan K, Yoon JH, Xu Y, deVries S, Camboni M, Janssen PM, Varki A, Martin PT (2010) A human-specific deletion in mouse Cmah increases disease severity in the mdx model of duchenne muscular dystrophy. Sci Transl Med 2:42–54

Chou HH, Takematsu H, Diaz S, Iber J, Nickerson E, Wright KL, Muchmore EA, Nelson DL, Warren ST, Varki A (1998) A mutation in human CMP-sialic acid hydroxylase occurred after the Homo-Pan divergence. Proc Natl Acad Sci U S A 95:11751–11756

Collins BE, Fralich TJ, Itonori S, Ichikawa Y, Schnaar RL (2000) Conversion of cellular sialic acid expression from N-acetyl- to N-glycolylneuraminic acid using a synthetic precursor, N-glycolylmannosamine pentaacetate: inhibition of myelin-associated glycoprotein binding to neural cells. Glycobiology 10:11–20

Diaz SL, Padler-Karavani V, Ghaderi D, Hurtado-Ziola N, Yu H, Chen X, Brinkman-Van der Linden EC, Varki A, Varki NM (2009) Sensitive and specific detection of the non-human sialic acid N-glycolylneuraminic acid in human tissues and biotherapeutic products. PLoS One 4:e4241

Feng L, Hong S, Rong J, You Q, Dai P, Huang R, Tan Y, Hong W, Xie C, Zhao J, Chen X (2013) Bifunctional unnatural sialic acids for dual metabolic labeling of cell-surface sialylated glycans. J Am Chem Soc 135:9244–9247

Fernandez-Marrero Y, Hernandez T, Roque-Navarro L, Talavera A, Moreno E, Grinan T, Vazquez AM, de Acosta CM, Perez R, Lopez-Requena A (2011) Switching on cytotoxicity by a single mutation at the heavy chain variable region of an anti-ganglioside antibody. Mol Immunol 48:1059–1067

Ghaderi D, Taylor RE, Padler-Karavani V, Diaz S, Varki A (2010) Implications of the presence of N-glycolylneuraminic acid in recombinant therapeutic glycoproteins. Nat Biotechnol 28:863–867

Ghosh S, Roseman S (1965) The sialic acids. V. N-acyl-D-glucosamine 2-epimerase. J Biol Chem 240:1531–1536

Harduin-Lepers A, Vallejo-Ruiz V, Krzewinski-Recchi MA, Samyn-Petit B, Julien S, Delannoy P (2001) The human sialyltransferase family. Biochimie 83:727–737

Hayakawa T, Aki I, Varki A, Satta Y, Takahata N (2006) Fixation of the human-specific CMP-N-Acetylneuraminic acid hydroxylase pseudogene and implications of haplotype diversity for human evolution. Genetics 172:1139–1146

Hedlund M, Tangvoranuntakul P, Takematsu H, Long JM, Housley GD, Kozutsumi Y, Suzuki A, Wynshaw-Boris A, Ryan AF, Gallo RL, Varki N, Varki A (2007) N-glycolylneuraminic acid deficiency in mice: implications for human biology and evolution. Mol Cell Biol 27:4340–4346

Hedlund M, Padler-Karavani V, Varki NM, Varki A (2008) Evidence for a human-specific mechanism for diet and antibody-mediated inflammation in carcinoma progression. Proc Natl Acad Sci U S A 105:18936–18941

Higashi H, Hirabayashi Y, Fukui Y, Naiki M, Matsumoto M, Ueda S, Kato S (1985) Characterization of N-glycolylneuraminic acid-containing gangliosides as tumor-associated Hanganutziu-Deicher antigen in human colon cancer. Cancer Res 45:3796–3802

Irie A, Koyama S, Kozutsumi Y, Kawasaki T, Suzuki A (1998) The molecular basis for the absence of N-glycolylneuraminic acid in humans. J Biol Chem 273:15866–15871

Lofling JC, Paton AW, Varki NM, Paton JC, Varki A (2009) A dietary non-human sialic acid may facilitate hemolytic-uremic syndrome. Kidney Int 76:140–144

Macauley MS, Chan J, Zandberg WF, He Y, Whitworth GE, Stubbs KA, Yuzwa SA, Bennet AJ, Varki A, Davies GJ, Vocadlo DJ (2012) Metabolism of vertebrate amino sugars with N-Glycolyl groups: intracellular β-O-linked N-glycolylglucosamine (GlcNGc), UDP-GlcNGc, and the biochemical and structural rationale for the substrate tolerance of β-O-linked β-N-acetylglucosaminidase. J Biol Chem 287:28882–28897

Malykh YN, Schauer R, Shaw L (2001) N-Glycolylneuraminic acid in human tumours. Biochimie 83:623–634

Nohle U, Schauer R (1984) Metabolism of sialic acids from exogenously administered sialyllactose and mucin in mouse and rat. Hoppe Seylers Z Physiol Chem 365:1457–1467

Padler-Karavani V, Hurtado-Ziola N, Pu M, Yu H, Huang S, Muthana S, Chokhawala HA, Cao H, Secrest P, Friedmann-Morvinski D, Singer O, Ghaderi D, Verma IM, Liu YT, Messer K, Chen X, Varki A, Schwab R (2011) Human xeno-autoantibodies against a non-human sialic acid serve as novel serum biomarkers and immunotherapeutics in cancer. Cancer Res 71:3352–3363

Padler-Karavani V, Tremoulet AH, Yu H, Chen X, Burns JC, Varki A (2013) A simple method for assessment of human anti-Neu5Gc antibodies applied to kawasaki disease. PLoS One 8:e58443

Pearce OM, Läubli H, Verhagen A, Secrest P, Zhang J, Varki NM, Crocker PR, Bui JD, Varki A (2014) Inverse hormesis of cancer growth mediated by narrow ranges of tumor-directed antibodies. Proc Natl Acad Sci U S A. 111(16):5998–6003

Pham T, Gregg CJ, Karp F, Chow R, Padler-Karavani V, Cao H, Chen X, Witztum JL, Varki NM, Varki A (2009) Evidence for a novel human-specific xeno-auto-antibody response against vascular endothelium. Blood 114:5225–5235

Pouilly S, Piller V, Piller F (2011) Metabolic glycoengineering through the mammalian GalNAc salvage pathway. FEBS J 279(4):586–598

Sarkar AK, Fritz TA, Taylor WH, Esko JD (1995) Disaccharide uptake and priming in animal cells: inhibition of sialyl Lewis X by acetylated Galβ1→ 4GlcNAcβ-O-naphthalenemethanol. Proc Natl Acad Sci U S A 92:3323–3327

Saxon E, Bertozzi CR (2000) Cell surface engineering by a modified Staudinger reaction. Science 287:2007–2010

Schauer R, Sommer U, Krüger D, Van UH, Traving C (1999) The terminal enzymes of sialic acid metabolism: acylneuraminate pyruvate-lyases. Biosci Rep 19:373–383

Tangvoranuntakul P, Gagneux P, Diaz S, Bardor M, Varki N, Varki A, Muchmore E (2003) Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid. Proc Natl Acad Sci U S A 100:12045–12050

Varki A, Schauer R (2009) Sialic acids. In: Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW, Etzler ME (eds) Essentials of glycobiology, 2nd edn. Cold Spring Harbor, New York

Varki NM, Strobert E, Dick EJ, Benirschke K, Varki A (2011) Biomedical differences between human and nonhuman hominids: potential roles for uniquely human aspects of sialic acid biology. Annu Rev Pathol 6:365–393