REVIEW

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Increasing the α 2, 6 Sialylation of Glycoproteins May Contribute to Metastatic Spread and Therapeutic Resistance in Colorectal Cancer

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Abnormal glycosylation due to dysregulated glycosyltransferases and glycosidases is a key phenomenon of many malignancies, including colorectal cancer (CRC). In particular, increased ST6 Gal I (β-galactoside α 2, 6 sialyltransferase) and subsequently elevated levels of cell-surface α 2, 6-linked sialic acids have been associated with metastasis and therapeutic failure in CRC. As many CRC patients experience metastasis to the liver or lung and fail to respond to curative therapies, intensive research efforts have sought to identify the molecular changes underlying CRC metastasis. ST6 Gal I has been shown to facilitate CRC metastasis, and we believe that additional investigations into the involvement of ST6 Gal I in CRC could facilitate the development of new diagnostic and therapeutic targets. This review summarizes how ST6 Gal I has been implicated in the altered expression of sialylated glycoproteins, which have been linked to CRC metastasis, radioresistance, and chemoresistance. **(Gut Liver 2013;7:629-641)**

Key Words: Colorectal neoplasms; Beta-D-galactoside alpha 2-6-sialyltransferase; Neoplasm metastasis; Radioresistance; Chemoresistance

INTRODUCTION

Glycosylation, which is the most frequent posttranslational protein modification, can affect the folding, stability, and functions of proteins.[1] Numerous genes in the human genome encode glycan-synthesis-related proteins,[2,3] which are responsible for generating the sugar chains that are linked to proteins in order to form glycoproteins; these sugars are classified as either N-linked glycan chains,[4] which are bound to the amidic nitrogens of asparagines, or O-linked glycan chains,[5] which are attached to the hydroxyl groups of serines or threonines. Cellular transformation is typically accompanied by alterations in the composition of glycoproteins, which are major constituents of the cell membrane, and abnormal glycosylation has been correlated with cancer progression and malignancy.[6-9] In particular, heavily sialylated N-glycans appear to be highly correlated with cancer metastasis.[10-12] In humans, N-acetylneuraminic acids (Neu5Ac) are the most prominent form of sialic acid in N-glycan chains; these negatively charged monosaccharides are widely distributed as terminal sugars that coat the eukaryotic cell surface and participate in various biological processes, including cell-cell communications, inflammation, immune defense, and cancer metastasis.[13] Generally, sialic acid is the name for the most common type of this group, Neu5Ac. Due to the important biological and cancer-related functions of these glycans, numerous researchers have studied the biosynthesis, acceptor substrate transfer, degradation, and recycling of sialic acids.[14-16]

Glycosylation requires the coordinated activity of various glycosyltransferases, which catalyze the transfer of monosaccharide residues from nucleotide sugar donors to specific acceptor substrates, forming glycosidic bonds. Sialyltransferases, which are key enzymes in the biosynthesis of sialic acid-containing glycoproteins and glycolipids, are a subset of glycosyltransferases that use cytidine monophosphate (CMP) to catalyze the transfer of sialic acids to the ends of the carbohydrate chains that are linked to glycoproteins or glycolipids.[14,17,18] Twenty members of the mammalian sialyltransferase family have been identified to date.[19,20] They are divided into four subfamilies according to their synthesized carbohydrate linkages: β-galactoside α 2, 3-sialyltransferases (ST3Gal I-VI); β-galactoside α 2, 6-sialyltransferases (ST6 Gal I and II); GalNAc α 2, 6-sialyltransferases (ST6 GalNAc I-VI); and α 2, 8-sialyltransferases (ST8Sia I-VI).

In our efforts to identify radiation-specific genes as biomark-

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ers, we showed for the first time that ST6 galactose (Gal) I is a candidate biomarker for detecting radiation exposure.[21] Several subsequent reports found that ST6 Gal I-deficient mice are immunosuppressed due to impaired B lymphocyte function, indicating that ST6 Gal I plays an important role in the immune system, potentially via B cell-receptor signaling.[22-25] Although most of the existing studies on sialyltransferase activity have focused on immune-mediated diseases, the absence of ST6 Gal I has been specifically correlated with carcinoma differentiation *in vivo*,[26] and ST6 Gal I has been shown to be critical to the development of colorectal cancer (CRC).[27-39] Indeed, the upregulation of this sialyltransferase is probably the basis for the increased α 2, 6 sialylation seen in CRC cells. Furthermore, many clinical and experimental studies have found positive correlations between high ST6 Gal I activity and the metastatic phenotypes of tumor cells.[8,31,33,40-42] However, relatively few studies have investigated the ST6 Gal I-induced sialylation of glycoproteins in CRC, and the effects of this parameter on metastasis and therapeutic response. In this review, we would like to draw attention to the potential involvement of ST6 Gal I-induced sialylation of glycoproteins in CRC metastasis, and suggest that sialylation might be a novel target for efforts to overcome chemoresistance and radioresistance in CRC.

Recent studies assessing the glycome for cancer biomarkers have focused on the involvement of glycosylation in cancer development,[9,43] but so far there is little direct evidence that sialylation plays a causative role in CRC. The cell surface is globally decorated with sialic acids, but their exact roles are not yet known, and the substrates of ST6 Gal I during the growth and metastasis of CRC remain to be investigated. Here, we summarize emerging evidence that implicates ST6 Gal I in the altered expression of sialylated glycoproteins, and examine the molecular links between aberrantly elevated sialylation and the metastasis, radioresistance, and chemoresistance of CRC.

THE IMPORTANCE OF SIALIC ACIDS IN THE GASTROINTESTINAL TRACT

Numerous N-glycans are linked to asparagines in Asn-X-Ser/Thr (X≠Pro) motifs, which are the hallmark of glycoproteins. Multiple N-glycosylation sites exist in glycoproteins, each of which has the potential to be modified by dozens of different N-glycan structures.[44] All N-glycans share a common core sugar sequence of Manα1–6(Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAcβ1-Asn-X-Ser/Thr and may be divided into three types: 1) the oligomannose type, in which only mannose (Man) residues are attached to the core; 2) the complex type (e.g., triantennary and tetra-antennary glycopeptides), in which the outer chains are composed of sialic acid, Gal, and N-acetylglucosamine (GlcNAc) residues; and 3) the hybrid type, in which two branches emerge from the core, one terminating in Man and one terminating in a sugar of the complex type.[4] The three types of N-glycan and their symbolic representations of each monosaccharide and linkage, the International Union of Pure and Applied Chemistry short code, and symbolic nomenclature with geometric shapes used in Consortium for Functional Gly-

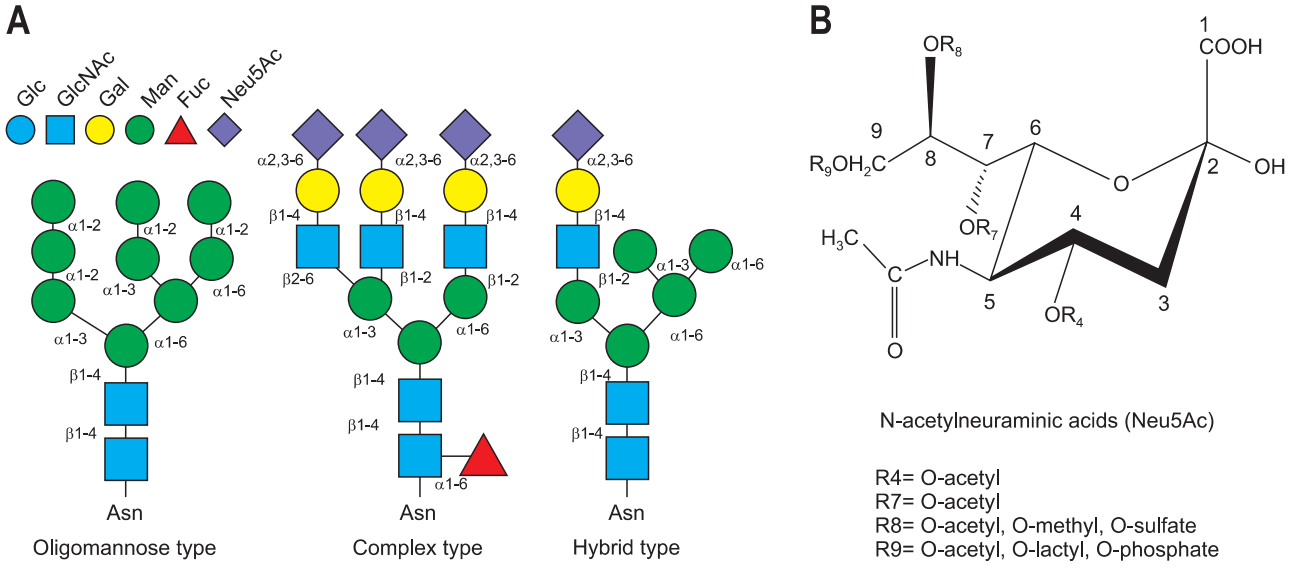

Fig. 1. Types of N-glycans and the structure of N-acetyl neuraminic acid (Neu5Ac). (A) The N-glycans that may be added to proteins at asparagine (Asn) residues are of three general types: the oligomannose type, the complex type, and the hybrid type. Each N-glycan contains the common core: $Man_3GlcNAc_2Asn$. For symbolic representations of each monosaccharide and linkage, the International Union of Pure and Applied Chemistry short code, and symbolic nomenclature with geometric shapes used in Consortium for Functional Glycomics are shown. (B) Neu5Ac have nine carbons, a carboxylic acid residue at the 1 position, and a variety of linkages to the underlying sugar chain at the 2 position. Various substitutions at the 4, 7, 8, and 9 positions combine with the linkages to generate the wide diversity of sialic acids found in nature. Glc, glucose; Gal, galactose; GlcNAc, N-acetyl glucosamine; Man, mannose; Fuc, fucose.

comics[45] are shown in Fig. 1A.

Among the sugars, the sialic acids are a group of neuraminic acids (5-amido-3, 5-dideoxy-D-glycero-D-galacto-nonulosonic acids).[46] More than 50 sialic acid forms have been found in nature, including Neu5Ac (the most abundant form), nonhuman *N*-glycolylneuraminic acid (Neu5Gc), and 2-keto-3-deoxy-nonulosonic acid (or deaminoneuraminic acid) (Kdn).[46,47] Generally, sialic acid is also the name for the most common member of this group, Neu5Ac. Interestingly, tumors including CRC accumulates the nonhuman Neu5Gc and studies about diagnostic significance of antibodies against Neu5Gc are in progress.[10,48] Innovative studies performed over the few past decades, especially from the Varki and Schauer labs, have expanded our knowledge of sialic acids. These monosaccharide are typically found as terminal components attached to the N- and O-linked chains of glycoproteins and glycolipids.[10,12,49,50] These negatively charged, acidic sugars share a nine-carbon backbone; they are hydrophilic; and they have a wide diversity that arises through the formation of various linkages to the 2-position of the underlying sugar chain and the potential for different substitutions at the 4, 7, 8, and 9 positions (Fig. 1B).[50] The biosynthesis of these sugars is very important, as is their ability to interact with high specificity and selectivity with carbohydrate-binding proteins (e.g., lectins, antibodies, receptors, and enzymes). Given their ability to end-cap glycan chains and their ubiquitous expression, sialic acids can regulate various physiological and pathological processes.[10] Particularly, the lining of the gastrointestinal tract has a dense and rich array of sialic acids, with are both displayed on the cell surface and secreted as glycoproteins.[51] Also, they exist in cellular secretions; in mucin, for example, sialic acids increase viscosity and shield the gastrointestinal epithelia from numerous pathogens.[10,51,52] As compared with sialomucin released in gastrointestinal tract, proteolytically secreted CRC mucins bearing abnormal forms of sialylation can be used as novel tumor marker for diagnosis and prognosis.[53,54]

The level of sialic acids in the stomach tends to be very low,

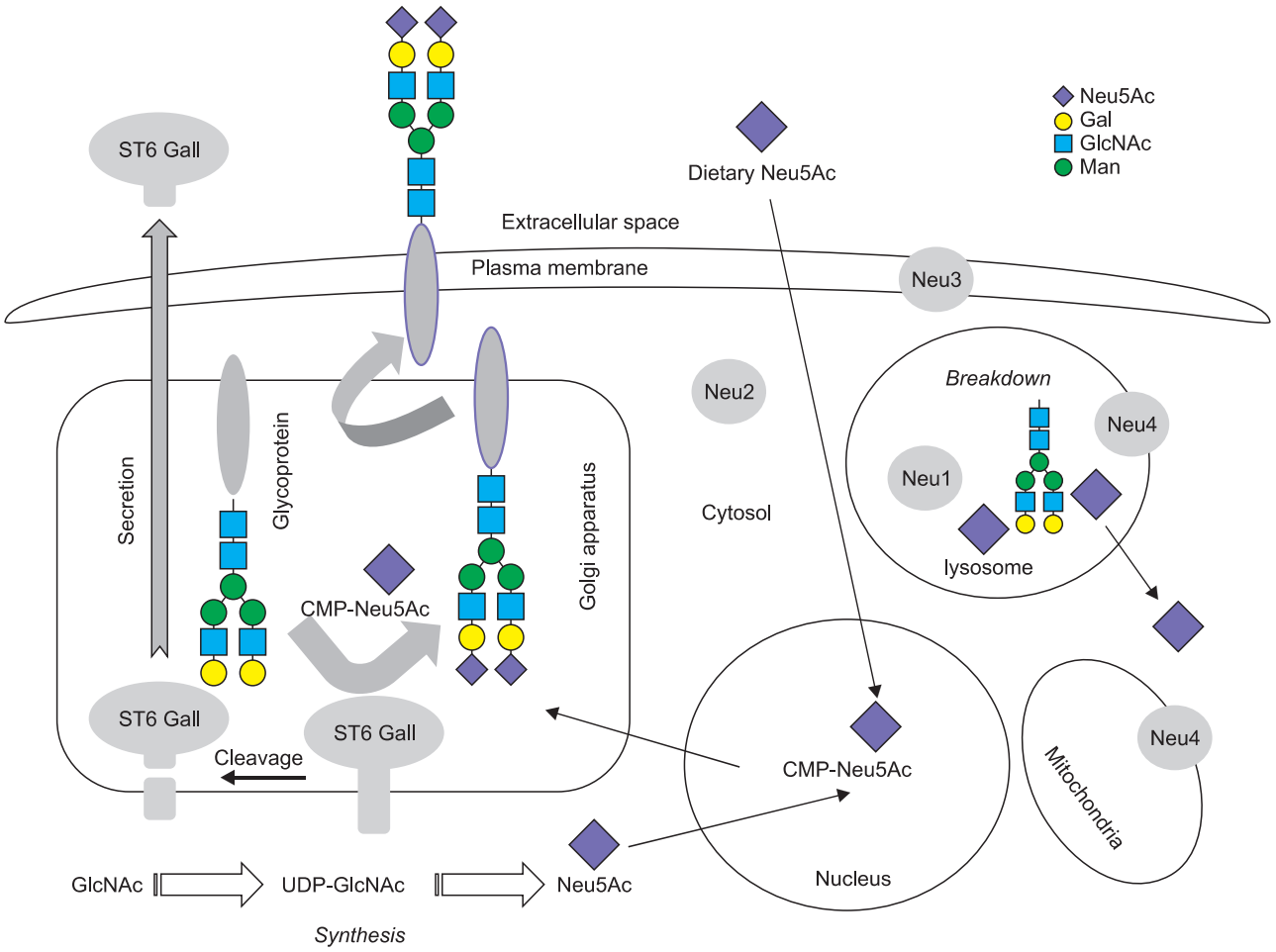

Fig. 2. Schematic representation of the sialic acid metabolism pathway in eukaryotic cells. The synthesis, activation, conjugation, and breakdown of mammalian sialic acids (N-acetyl neuraminic acid, Neu5Ac) are shown. Sialic acid is taken in through the diet or synthesized in the cytosol. From there, it is transported into the nucleus, where cytidine monophosphate (CMP)-sialic acid is synthesized. In the Golgi apparatus, ST6 Gal I transfers the sialic acid from CMP-sialic acid to a glycoprotein. ST6 Gal I is cleaved by cathepsin-like proteases, and then released from the cell. The sialylated conjugates can be cleared by neuraminidases in lysosomes, the cytosol, the plasma membrane, and mitochondria. Gal, galactose; GlcNAc, N-acetyl glucosamine; Man, mannose; Neu, neuraminidase.

and they are often replaced by sulfation; however, there are sufficient sialic acids in the stomach to act as receptors for a wide variety of pathogens that facilitate gastric inflammation and tumor formation.[55-57] Further down in the gastrointestinal tract the sialic acids are usually highly modified, often via the addition of O-acetyl esters. Compared with the stomach and small intestine, the highest density of O-acetylated sialic acids is found in the colon, which harbors di-O-acetylated and even tri-O-acetylated sialic acids.[58,59] Notably, this acetylation tends to decrease during the development of CRC.[60,61] We do not yet know why such modification is more common in the colon or why it decreases in CRC. Thus, future studies are needed to explore the roles of acetylated sialic acids in the gastrointestinal tract, the underlying molecular mechanisms, the involved acetyltransferases, and the functional significance of sialic acids in the development of CRC.

SYNTHESIS, TRANSFER, AND ELIMINATION OF SIALIC ACIDS

Sialic acids are synthesized from GlcNAc via a multistep process that involves many enzymes and occurs in the cytosol. Both synthesized and dietary sialic acids are transported into the nucleus, where CMP-Neu5Ac synthetase acts on them to form CMP-Neu5Ac. This is then transported to the Golgi apparatus to be used for the sialyltransferase-mediated formation of glycoconjugates, which are subsequently secreted or anchored to the cell membrane (Fig. 2).[14] Among the various sialyltransferases, β-galactoside α2, 6 sialyltransferase (ST6 Gal I; EC 2.4.99.1) transfers sialic acids (Neu5Ac) from the donor substrate, cytidine-5`-monophospho-N-acetyneuraminic acid (CMP-β-NeuAc), to the terminal Gal residues of N-glycans to create an α 2, 6 linkage.[29] This enzyme is localized in the Golgi apparatus in a membrane-bound form, and is transformed into the cleaved, catalytically active form by β secretase-like proteases (Fig. 2).[62,63]

Sialyltransferases were initially isolated and characterized from various tissues and fluids, including the liver, brain, thyroid, mammary gland, saliva, colostrum, and serum.[64-72] Other studies found that plasma sialyltransferase levels were reported to be elevated in CRC patients.[66,73] Based on these findings, researchers speculated that sialyltransferases could function as tumor biomarkers, and suggested that plasma sialyltransferase levels could be used to measure cancer progression, metastasis, and/or treatment responses. In addition, patients with metastasizing tumors have high serum levels of ST6 Gal I that have been correlated with the progression and metastasis of CRC.[74] However, the biological role of ST6 Gal I in the plasma has not yet been elucidated. While the Golgi appears to be the main site of ST6 Gal I-mediated sialylation in most cells, the detection of soluble ST6 Gal I in body fluids and media from cultured cells suggests that secretory ST6 Gal I may have other functions.[75] Alternatively, secretory ST6 Gal I could simply arise from the secretion of Golgi-anchored ST6 Gal I from cancer cells when its enzymatic activity is no longer required. Other glycosyltransferases have been detected in the systemic circulations of cancer patients, where they have been correlated with disease progression and poor prognosis.[76,77] Because these circulating enzymes do not have access to sufficient donor nucleotide sugars, they should be unable to confer extracellular transferase activity. The biological significance of these secretory enzymes remains a mystery, but researchers have speculated that they could confer a lectin-like activity by recognizing their acceptor substrates and/or could contribute to clearing sugar nucleotides from body fluids.[78,79] Regardless, the existing studies collectively suggest that secretory ST6 Gal I could be a biomarker for the clinical evaluation of CRC, highlighting the need to fully elucidate the function of this enzyme.

Contrary to the enzymatic activity of sialyltransferases, neuraminidase (which is also called N-acetylneuraminosyl glycohydrolase or sialidase) is a member of the glycosidase family, and is responsible for catalyzing the hydrolytic cleavage of sialic acid from the oligosaccharide chains of its glycoconjugates.[6,80] There are at least four types of neuraminidase: lysosomal neuraminidase (Neu1), cytosolic neuraminidase (Neu2), plasma membrane-associated neuraminidase (Neu3), and lysosomal/mitochondrial membrane-associated neuraminidase (Neu4) (Fig. 2). Seminal studies by Miyagi and coworkers showed that the four types of mammalian neuraminidase have different substrate specificities and behave in different manners during the progression of CRC.[6,11,40,80-85] For example, Neu1 contributes to suppressing CRC metastasis,[82] while Neu3 is markedly up-regulated in colon cancer and functions in colon carcinogenesis.[85] Although the ST6 Gal I-mediated α 2, 6 sialylation of glycoconjugates has been observed only in the Golgi, elimination of sialic acids has been found in the lysosome, plasma membrane, cytosol, and mitochondria. Thus, future studies are warranted to examine how ST6 Gal I and the different neuraminidases could aberrantly regulate α 2, 6 sialylation in CRC.

ST6 GAL I ACTIVITY IN CRC

The progression of CRC typically follows a slow and stepwise process of accumulating mutations under the influence of environmental and epigenetic factors.[86-89] Approximately 20% of all CRC cases have familial or congenital mutations in genes that increase colon cancer risk; these individuals tend to develop the disease earlier. The remaining cases (80%) are sporadic and tend to develop later in life, suggesting the involvement of environmental factors and the long-term accumulation of multiple genetic mutations and/or epigenetic alterations. The model of CRC development proposed by Fearon and Vogelstein[90] in 1990 has been updated, and recent research has advanced our understanding of this multistep process.[90-92] Briefly, normal colon epithelia acquire certain mutations, develop into hyperplasias

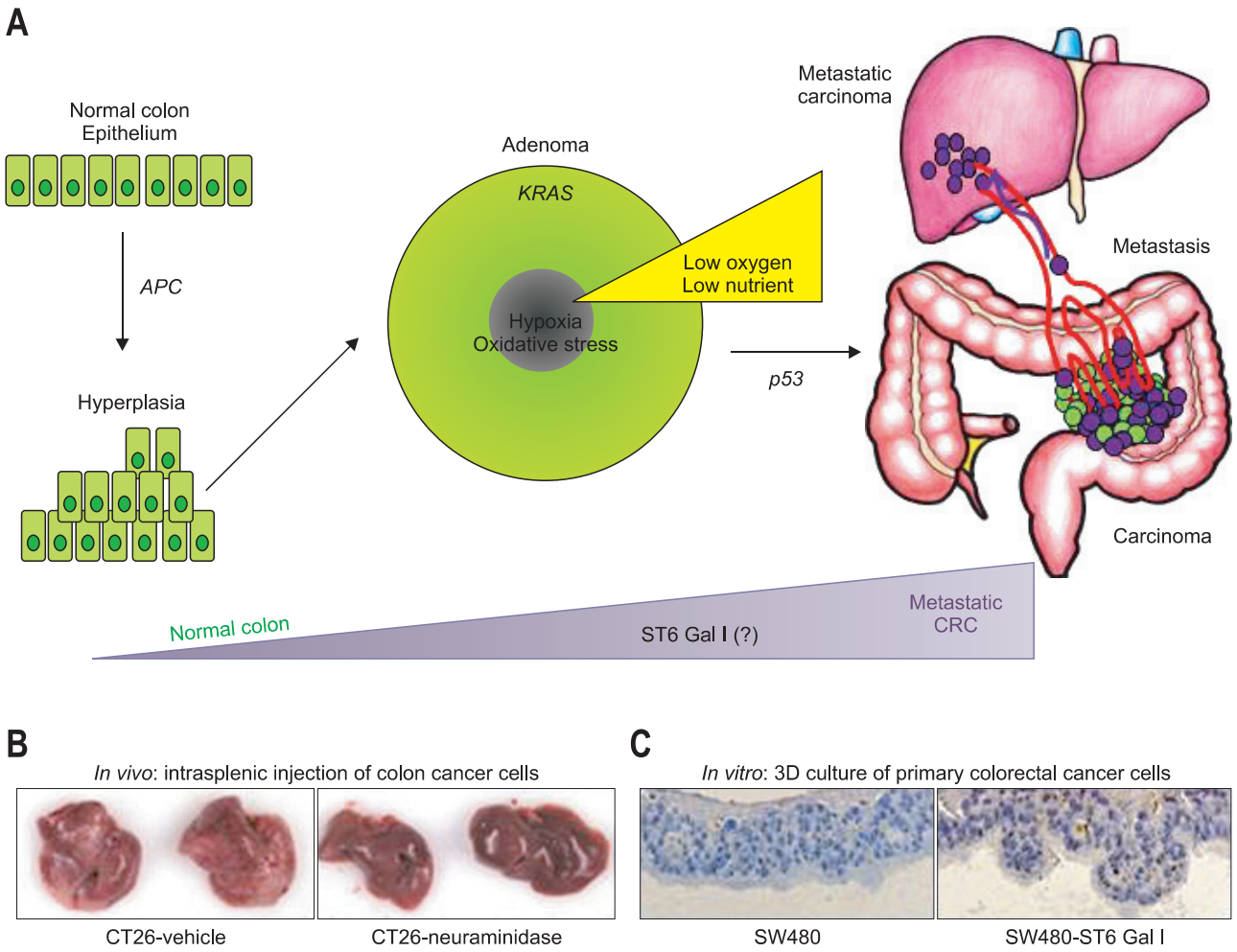

Fig. 3. ST6 galactose (Gal) I activity may be increased during the development of colorectal cancer (CRC). CRC arises through a series of well-characterized molecular and histopathologic changes that transform normal colonic epithelial cells into metastatic carcinoma cells. (A) Mutations in *APC* are required for tumor initiation, and the subsequent progression from adenoma to metastatic carcinoma is accompanied by genomic instability and sequential mutations in *KRAS* and *p53*, and other genes. (B) *In vivo* experimental results, splenic injections of CT26 cell treated with or without neuraminidase were used to examine hepatic metastasis of mouse CRC cells. (C) *In vitro* 3-dimensional (3D)-culture experiments show that ST6 Gal I-overexpressing SW480 human primary CRC cells are more invasive than control cells.

and then progress to adenomas. Inactivation of the tumor suppressor, adenomatous polyposis coli (APC), is a central event that is uniformly observed in early adenomas.[93] APC mutations have been associated with genomic instability, which appears to begin in the early stages of CRC development.[94] Subsequently, adenomas develop into carcinomas via an aneuploid pathway that involves mutations in p53 and KRAS, chromosomal instability, microsatellite instability, and a gain of chromosome 8q (Fig. 3A). The high mortality rate of CRC is mainly due to its dissemination to secondary organ sites. This requires additional changes, such as the loss of chromosome 8p, and the transition from carcinoma to metastatic CRC typically occurs over years as these changes accumulate.[95] Metastasis, which represents the final step in CRC progression, involves migration, invasion, anoikis resistance, extravasation into the liver and/or lung, and angiogenesis.[96] A variety of molecules contribute to this process, including various key cell surface proteins that bear complex N-glycan arrays. It has long been appreciated that alterations in cell surface glycans can contribute to the transformation and metastatic properties of tumor cells.[7] In particular, distinct changes of sialylation and sialylated structures have been associated with tumor malignancy.[8,10,41]

With respect to the correlation between ST6 Gal I and CRC, overexpression of ST6 Gal I has been observed in CRC and other human cancers, including gastric, breast, ovarian and cervix, as well as leukemia.[27,97-102] This increases the α 2, 6 sialylated N-glycan structures found at the surfaces of cancer cells, which has been positively correlated with metastasis and poor prognosis.[8] Furthermore, *in vitro* and *in vivo* studies have suggested that ST6 Gal I may play key roles in cancer cell adhesion, migration, invasion, and differentiation in CRC.[26,34,45,103-105] In agreement with these reports, we obtained similar findings. A mouse CRC cell line (CT26 cells) was incubated with or without purified neuraminidase (to remove all sialic acids from the cell

membrane) and injected into the spleens of mice, and liver metastasis of CRC cells was assessed *in vivo*. As shown in Fig. 3B, hepatic metastasis of the CT26 cells was completely blocked by removal of the surface sialic acids (unpublished data). Furthermore, consistent with previous results from *in vitro* adhesion and migration experiments,[103,104] we found that ST6 Gal I-overexpressing SW480 human CRC cells showed critically invasive structures, as characterized by multicellular outgrowth into collagen gels in 3-dimensional collagen cocultured with human dermal fibroblasts (unpublished data) (Fig. 3C).

These findings support the hypothesis that ST6 Gal I could facilitate the progression (particularly metastasis) of CRC. However, we do not yet know: 1) the factors responsible for transcriptionally or translationally controlling ST6 Gal-I expression in CRC; 2) the specific substrates of this sialyltransferase and the functional consequences of α 2, 6 sialylation; 3) whether (in the context of the CRC development model) APC, KRAS, and p53 mutations can alter ST6 Gal I expression (notably, a connection has been reported between ST6 Gal I and KRAS; see below); 4) whether the microenvironment found within rapidly growing solid tumors (i.e., hypoxia, nutrient insufficiency, and low pH) can influence ST6 Gal I activity; and 5) why O-acetylated sialic acids decrease with the progression of CRC. We are not yet able to describe a general role for ST6 Gal I in the diverse cellular events of CRC progression and metastasis. Additional studies are needed to determine how ST6 Gal I expression is regulated and how its enzymatic activity contributes to CRC development and metastasis.

COULD ST6 GAL I BE THE KEY TO EFFECTIVE ANTICANCER THERAPY AGAINST CRC?

CRC, which is the third most common malignancy worldwide,[106] is typically treated by surgical resection with or without adjuvant chemotherapy and radiotherapy to improve survival. Up to 20% of CRC patients will experience metastasis; among them, the 5-year survival rate is less than 10%, due to failure of treatment response.[107] Therefore, we critically need new studies into the mechanisms underlying the metastasis of CRC.

Various glycoproteins have been shown to enhance the progression of CRC to incurable metastasis;[8] among them are the integrins, which are essential for cell adhesion to the extracellular matrix (ECM) and the migration, invasion, and survival of cancer cells.[108] Integrins are glycosylated transmembrane adhesion receptors that are characterized by a large extracellular domain that contains multiple N-glycosylation sites and act as a linker.[109] Integrins expression has been correlated with metastatic CRC, and integrins can be used as prognostic factors for CRC.[110] In recent decades, many important discoveries from Bellis and coworkers have shown that the activity levels and substrates of ST6 Gal I are highly correlated with CRC development, CRC cell stemness, and therapeutic resistance. For example, ST6 Gal I-induced sialylation of integrin β1 was associated with CRC by Seales *et al*.[31] Furthermore, the Ras oncogene was shown to transcriptionally up-regulate ST6 Gal I expression, leading to over-sialylation of integrin β1 (but not integrin β3 or -β5) in colon epithelial cells; this Ras-induced α 2, 6 sialylation was found to critically regulate integrin β1 adhesion activity, thereby altering cell motility.[111] Similarly, KRAS-mediated transformation dramatically increased ST6 Gal I mRNA levels, enhancing the cell-surface α 2, 6 sialylation of NIH3T3 cells.[112] This suggests that KRAS-induced ST6 Gal I up-regulation and the subsequent enhancement of integrin β1 α 2, 6 sialylation may be involved in the development of CRC. We propose that ST6 Gal I-induced hyper-sialylation of integrin β1 promotes the metastasis of CRC by altering the cell's preference for the ECM and stimulating adhesion, migration, and invasion. In the context of cell survival, ST6 Gal I-induced sialylation of integrin β1 has been shown to protect cancer cells from apoptosis by blocking the adhesion of cells to galectin-3.[113,114] Moreover, α 2, 6 sialylation of the Fas death receptor was found to decrease CRC cell apoptosis,[39] and up-regulation of ST6 Gal I has been observed in stem cells of CRC.[27] Collectively, these diverse results suggest that ST6 Gal I may critically regulate CRC progression.

Integrin β1 is an adhesion receptor that is well known to heterodimerize with other integrin family members and participates in various cellular processes by forming focal adhesion complexes. These large complexes comprise integrins along with various combinations of some 150 signaling molecules that transduce integrin-mediated cellular survival signaling.[115] In the fields of radiobiology and experimental radiation oncology, the involvements of integrin β1 in the responses to radiotherapy and chemotherapy have been highlighted by Cordes and Park[116] and coworkers.[117] Radiotherapy is well known to induce both DNA damage responses and persistent tissue microenvironment remodeling, both of which contribute to radiation resistance.[118,119] The widespread phenomenon of cell-adhesion-mediated radioresistance has been recognized in various cancers, including CRC.[108,120-123] Hence, it has been suggested that integrin β1 may mediate essential survival signals following radiotherapy, and that integrin β1-blocking reagents could act as radiosensitizers in various solid tumors. Consistent with this, we demonstrated that ionizing radiation increases the levels of ST6 Gal I and α 2, 6 sialylated integrin β1 in human CRC cells, and enhances the adhesion and migration of these cells via integrin β1-mediated cellular survival signaling.[103,104,124] These findings strongly suggest that ST6 Gal I-induced sialylation of integrin β1 and the subsequent activation of focal adhesion molecules (e.g., p130CAS, focal adhesion kinase, paxillin, and Src) and AKT signaling may be involved in radioresistance (Fig. 4).

Despite recent advances in chemotherapy, the available anticancer drugs have not improved the prognosis of CRC, and approximately 30% of patients experience recurrence.[125-127] In general, the chemotherapeutic regimens for metastatic CRC consist

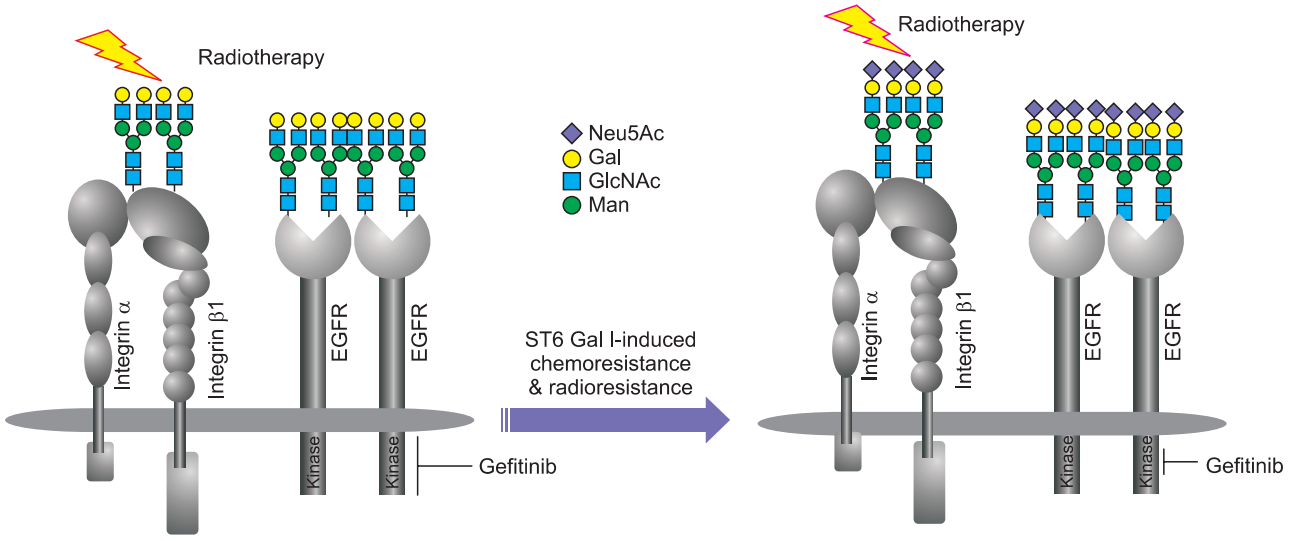

Fig. 4. Hypothetical pathway(s) for ST6 galactose (Gal) I-induced radioresistance and chemoresistance in colorectal cancer. EGFR, epidermal growth factor receptor; Neu5Ac, N-acetyl neuraminic acid; GlcNAc, N-acetyl glucosamine; Man, mannose.

of fluorouracil derivatives with irinotecan, or oxaliplatin (called FOLFIRI and FOLFOX, respectively).[128] The clinical management of CRC was critically improved by the discovery of KRAS mutations in CRC patients and studies on the use of monoclonal antibodies targeting epidermal growth factor receptor (EGFR).[129,130] Although patients with activating mutations in the KRAS are typically resistant to EGFR-targeted chemotherapy, those with wild-type KRAS typically respond to monoclonal antibodies against EGFR.[131,132] Therefore, treatments may be designed based on the KRAS status of patients with advanced CRC.

EGFR, which is widely expressed in the gastrointestinal tract, is frequently overexpressed in CRC compared with normal intestinal epithelia. It increases the metastatic potential of CRC cells and has been associated with poor prognosis.[133,134] EGFR is a transmembrane glycoprotein that has an extracellular ligand binding domain and an intracellular tyrosine kinase domain; it acts as a receptor tyrosine kinase and mediates cellular responses to growth factor through the RAS, signal transduction and activator of transcription, and PI3K/AKT pathways.[135] Studies have shown that the loss of APC, which is sufficient to initiate the formation of intestinal polyps in experimental animals, is associated with increased EGFR activity in CRC.[136,137] However, studies on EGFR-targeting monoclonal antibodies in CRC patients failed to show a relationship between EGFR expression and the response to chemotherapy.[135,138,139] EGFR mutations have been found in nonsmall cell lung cancer patients who showed responses to the EGFR tyrosine kinase inhibitors (TKIs), gefitinib, and erlotinib,[140] suggesting that EGFR-targeting therapies could be useful against cancers that harbor activating mutations in the intracellular kinase domain of EGFR. Such mutations are rare in CRC, however, and CRC does not typically respond to gefitinib. Similarly, sequencing of the EGFR exons in human CRC cell lines (e.g., SW620, SW480, CaCo-2, HCT116, and HT-29) failed to identify any mutation of EGFR.[141] The sensitivity of CRC to EGFR TKIs was not found to be dependent upon p53 status, which is an important determinant for the responsiveness to fluorouracil derivatives and oxaliplatin.[142] Interestingly, the localization of EGFRs was shown to alter the responsiveness of cancer cells to gefitinib, with membrane localization of EGFR appearing to support EGFR TKI resistance.[143] Therefore, future studies are warranted to examine downstream events and regulatory mechanisms, in the hopes of improving the efficacy of EGFR-targeting treatments in CRC.

Most studies on EGFR have focused on its amplification, activating mutations, and the development of EGFR-targeting drugs;[135] in contrast, fewer studies have examined the regulation of EGFR activity via posttranslational modifications, including glycosylation. Moreover, no useful biomarkers for anti-EGFR therapy have yet been identified, and activating EGFR mutations are the only reliable predictors for responsiveness to EGFR inhibitors. The Taniguchi and Nishio groups both reported that fucosylation of EGFR at 10 N-glycosylation sites of its extracellular domain was essential for EGFR activity and EGFR-mediated survival signaling.[144,145] Thereafter, the Wong group reported that EGFR is hypersialylated in human lung cancer; furthermore, higher sialylation was found in invasive lung cancer, and sialylation was shown to regulate EGF-mediated receptor dimerization and EGFR phosphorylation in lung cancer cells.[146] The authors identified various sialylated glycoproteins (including EGFR) in human lung cancer, and performed site-specific glycoform mapping and glycan sequencing. However, although these reports showed that N-glycosylations (e.g., sialylation and fucosylation) can regulate EGFR activity, researchers have not yet fully examined the enzymes responsible for sialylating EGFRs in the context of ST6 Gal I-overexpressing CRC or assessed the effects of EGFR-targeting drugs on sialylated EGFR in human

cancers.

Particularly, we found that ST6 Gal-I induces α 2, 6 sialylation of EGFR in human CRC cell lines. The absence of ST6 Gal I activity promoted cell proliferation and tumor growth *in vitro* and *in vivo*, and the anticancer efficacies of the EGFR-TKI, gefitinib, were significantly increased and decreased in ST6 Gal-I-deficient and -overexpressing CRC cell lines, respectively.[147] To our knowledge, this is the first report suggesting that ST6 Gal-I-mediated hypersialylation of EGFR can affect CRC cell growth and the sensitivity of EGFR TKI resistant–CRC cells to gefitinib (Fig. 4). Thus, along with activating mutations and up-regulation of EGFR, the α 2, 6 sialylation of EGFR could represent a reliable biomarker for clinical responses to anti-EGFR therapy. In the future, gaining a full understanding of sialylated EGFR and its dysregulation in CRC may facilitate new advances in the management of this cancer type.

IMPLICATIONS AND FUTURE DIRECTIONS

Unlike proteins, glycans are not fixed in the genome and do not depend on a template for their biosynthesis. Instead, the structures of the glycan chains that cover glycoproteins result from the concordant activity of ~250 to 300 enzymes, including glycosyltransferases.[3,148-150] Moreover, the spatiotemporal distributions of these vast arrays of carbohydrate structures depend on the concentrations and locations of high-energy nucleotide sugar donors, which are substantially regulated by energy metabolism. In the context of posttranslational modification and cancer metabolism, glycosylation (including sialylation) is known to play a crucial role at the interface between metabolism and signaling in cancer.[151] A number of carbohydrate-related genes, including those encoding fucosyltransferase VII, β-galactoside α-2, 3-sialyltransferase I (ST3 Gal I), and UDP-Gal transporter-1, were shown to be induced by hypoxic culture of human CRC cells.[152] Moreover, hypoxia-mediated increase of sialyl Lewis antigens were observed in CRC cells to adhere to endothelial cells via selectin-integrin interaction and finally to metastasize to secondary organ.[83,152] Hypoxia crucially influences the clinical outcome of radiotherapy; hypoxic cells are resistant to radiation treatment[153] and low oxygen concentrations tend to increase the proportion of cancer stem cells.[154-157] Therefore, future work is warranted to examine whether ST6 Gal I could be involved in the Warburg effect, perhaps explaining its involvement in the hypoxia-induced metabolic shift, metastatic potential, angiogenesis, and radioresistance of CRC.

Many early studies sought to characterize the sialylation changes associated with oncogenic transformation and identify tumor-associated sialylated glycoproteins. More recently, researchers have addressed the functional significance of aberrant sialylation in CRC metastasis. The α 2, 6 sialylation of membrane-anchored receptors has been shown to modulate CRC cell adhesion, motility, and invasiveness. Moreover, sialic-acid-containing cell surface glycoproteins have been found to affect chemosensitivity and radiosensitivity.[8,124,147] As sialic acids are common outer components of the glycan structure, where they may protect CRC cells from extracellular stresses capable of affecting cell fate, we and other researchers have proposed that inhibitors specific for the sialyltransferases associated with numerous human cancers could be therapeutically relevant.[158,159] A number of studies have suggested that sialyltransferase inhibitors may play roles in tumor growth and metastasis. Unfortunately, most of the candidate inhibitors that have been developed have failed to provide the specificity or ease of use needed for clinical applications. Examples include AL10, a lithocholic acid analog that was derived from soyasaponin I by chemical synthesis but was found to act as a pan-sialyltransferase inhibitor,[160] and soyasaponin I, which functions as a β-galactoside α-2, 3-sialyltransferase I (ST3 Gal I) inhibitor, but is not amenable to large-scale purification.[161] Recently, Paulson's group developed a high-throughput screening method for identification of sialyltransferase and fucosyltransferase inhibitors.[162] They screened 16,000 compounds against five different glycosyltransferases (ST6 Gal I, FUT6, FUT7, ST3 Gal I, and ST3 Gal III), and identified several candidate inhibitors. However, no small molecule inhibitor specific for ST6 Gal I has yet been identified. Sialyltransferases (as with other glycosyltransferases) have narrow specificities for both donor and acceptor substrates, which are likely to be recognized strictly by the binding region. Therefore, properly designed inhibitors could be expected to have specific and potent activities. It should be noted that some reports have suggested that sialylation can also have negative effects on cancer progression, such as the apparent inhibitory effect of N-glycan α 2, 6 sialylation on glioma formation.[163,164] However, although sialylated glycoproteins may have much more multifaceted and sophisticated functions than initially thought, they generally appear to have positive effects on tumorigenesis, metastasis, and chemoresistance.

In sum, we believe that ST6 Gal I may turn CRC into a supersurvivor against radiotherapy and chemotherapy. At present, a few glycan-targeting anticancer strategies are being investigated in clinical trials.[165] As we learn more about the roles of sialylated glycans in CRC progression and metastasis, novel targets will continue to emerge.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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