Sialic Acids Sweeten a Tumor’s Life

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Abstract
Over four decades ago, specific tumor characteristics were ascribed to the increased expression of sialic acid sugars on the surface of cancer cells, and this led to the definition of sialic acids as potential therapeutic targets. Recent advances in glycobiology and cancer research have defined the key processes underlying aberrant expression of sialic acids in cancer, and its consequences, more precisely. These consequences include effects on tumor growth, escape from apoptosis, metastasis formation, and resistance to therapy. Collectively, these novel insights provide further rationale for the design and development of therapeutic approaches that interfere with excessively high expression of sialic acids in cancer cells. Strategies to target aberrant sialylation in cancer, however, have evolved comparatively slowly. Here, we review recent findings that emphasize the detrimental effects of hypersialylation on multiple aspects of tumor growth and behavior. We also discuss novel therapeutic strategies. Cancer Res; 74(12); 1–6. ©2014 AACR.

Introduction
Tumor cells of various origins feature increased expression of sialic acid sugars on membrane glycoproteins and glycolipids and their secretion into the tumor microenvironment. Sialic acids are synthesized in and expressed by essentially every vertebrate cell, and are involved in multiple different physiologic processes. However, hypersialylation of tumor cells relative to their untransformed normal counterparts specifically benefits tumor cell growth and correlates with a poor prognosis for patients with cancer (1, 2). Sialic acids comprise a family of more than 50 carbohydrates that share a nine-carbon backbone (C1-9) to which specific chemical modifications are enzymatically attached inside the cell. The most common sialic acid derivative found in mammals is N-Acetylneuraminic acid (Neu5Ac) which bears an acetyl group on the C5. In general, sialic acids terminate the outer end of glycans (sialoglycans), where they are enzymatically linked to other carbohydrates, such as the monosaccharide galactose, by glycosidic bonds. This enzymatic process is carried out by more than 20 distinct Golgi-resident sialyltransferases (ST) that link sialic acids via their second carbon (C2) to the carbon atom at position C3 (ST3Gal I-VI), C6 (ST6Gal I,II and ST6Gal-NAc I-VI), or to C8 (ST8Sia I-VI) of carbohydrates, yielding α2,3-, α2,6-, or α2,8-linked sialic acids, respectively (3). The latter enzymes are also involved in creating α2,8-linked polysialic acids (PSA). Together, the multifarious chemical substitutions, the different linkages to underlying sugars, and their spatial and temporal organization result in a tremendous diversity of sialoglycans, termed the sialome by Cohen and Varki (4).

Although we have just begun to understand the biology of the sialome and its implications in pathology, sialoglycans are known to regulate glycoprotein and glycolipid structure, stability, trafficking, and function. Moreover, their prominent position on the cell membrane allows sialoglycans to effectively participate in cell–cell and cell–extracellular matrix interaction, including adhesion, migration, and immune recognition (3). There are receptor families that specifically recognize sialoglycans such as selectins or sialic acid-binding immunoglobulin-like lectins (Siglecs). Selectins are expressed on endothelial cells and leukocytes and enable extravasation of immune cells to sites of inflammation and also allow hematogenous spread of cancer cells. Siglecs are expressed on most cells of the immune system and can transmit immunosuppressive signals upon binding to sialic acid ligands. Increased expression of siglec ligands by tumor cells could thus contribute to tumor immune evasion (5–7).

In line with their vital role in many physiologic processes, several lines of evidence imply that aberrant expression of sialic acids confers major advantages to tumor cells, ranging from inhibition of apoptosis to resistance to cancer therapy. For these reasons, strategies to block aberrant sialylation on tumors may be highly beneficial, not only to directly limit tumor growth, but also to enhance the effect of cancer therapeutics.

Mechanisms of Aberrant Sialylation in Cancer
To date, three key mechanisms have been reported to cause aberrant sialylation in cancer cells. First, overexpression and/or altered activity of sialyltransferases results in increased sialylation of glycans and expression of specific tumor-associated carbohydrate antigens (e.g., sLe^a^, STn, GD2, GD3, or PSA; refs. 8, 9). The proto-oncogenes, Ras and c-Myc, have been shown to control transcription of the sialyltransferases ST6Gal I and ST3Gal I, II, and IV, respectively. This has been reported to...
result in increased α2,6-sialylation of β1-integrin (Ras) and high expression of sLeα antigen (c-Myc), both facilitating tumor cell motility (10, 11). In addition, low oxygen levels and high hormone levels have been found to upregulate expression of sialyltransferases. Hypoxia eventually selects highly aggressive tumor cells and is associated with a poor prognosis in patients. Low oxygen levels were found to induce ST3Gal I expression and subsequently sLeα antigen synthesis in colon cancer cells, eventually favoring binding to selectins and entry into the blood stream (12). Hatano and colleagues demonstrated that in hormone-sensitive prostate cancer cells, androgens control transcription of ST3Gal II sialyltransferase by inducing promoter demethylation, resulting in high GD1α expression, a sialoganglioside involved in tumor progression (13). Multiple other studies have reported overexpression of sialyltransferases in tumor tissue indicating that sialyltransferase upregulation is a dominant mechanism underlying hypersialylation in cancer.

The second mechanism was proposed by Almaraz and colleagues, who provided evidence that the metabolic flux through the sialic acid synthesis pathway is enhanced in cancer cells due to increased substrate availability or overexpression of genes involved in sialic acid biosynthesis. They demonstrated that sialylation of glycoproteins increased dramatically when enhancing the flux rate through the sialic acid pathway by the addition of sialic acid precursors to cancer cells in vitro (14). Interestingly, mainly glycoproteins affiliated with extracellular matrix (ECM) interactions and cell migration were found to be hypersialylated, instead of gross upregulation of protein sialylation. Whether this is truly a tumor-specific phenomenon remains to be answered, but these data imply that metabolic changes in sialic acid biosynthesis in the tumor microenvironment can lead to hypersialylation of cancer cells, and therefore alter the expression of molecules involved in migration and metastasis.
Sialic Acids and Apoptosis Evasion in Cancer

Increased tumor cell sialylation caused by differential expression of endogenous sialidases has been put forward as a third mechanism. Sialidases can enzymatically cleave sialic acids from glycans and thereby regulate shedding, plasticity, and degradation of sialoglycans. At present, four human sialidases have been identified, located in the lysosome (NEU1, 4), cytosol (NEU2), or plasma membrane (NEU3). Expression of NEU1, 2, and 4 has been reported to be decreased in malignancies, leading to accumulation of sialoglycans in cancer cells. Interestingly, NEU3 is found to be upregulated in some cancer cell types, but the functional consequences on sialylation are not yet understood (15).

Although the molecular mechanisms responsible for hypersialylation are starting to be unveiled, many questions remain, for instance how sialic acid and sialoglycan synthesis or hydrolysis rates relate to the function of individual glycoproteins and glycolipids in the cell membrane. Another unresolved issue is whether hypersialylation can have a causative role in tumorogenesis or is a bystander effect of malignant transformation. Overexpression of ST3Gal I in cancer cells has been reported to be sufficient to drive tumorigenesis in a mouse model for breast cancer, and Swindall and colleagues found indications that ST6Gal I upregulation is associated with cancer stem cell maintenance (16, 17). These findings at least suggest that sialyltransferase overexpression may have a significant role in promoting tumorogenesis.

Sialic Acids and Apoptosis Evasion in Cancer

Resisting cell death is one hallmark of cancer cells (18). Mutations or downregulation of molecules involved in the Fas receptor–Fas ligand (FasR–FasL) apoptotic pathway are well-known mechanisms exploited by cancer cells to escape apoptosis. Swindall and Bellis discovered a novel strategy whereby tumor cells escape from Fas-mediated apoptosis; Hypersialylation of the Fas receptor was shown to disable apoptosis induction in cancer cells (19). They identified FasR, which serves as a substrate for ST6Gal I. Silencing expression of ST6Gal I in cancer cells enhanced Fas ligand–induced apoptosis, whereas overexpression of ST6Gal I hindered Fas-mediated apoptosis. Detailed analysis revealed that α2,6-sialylation of the FasR prevents the initiation of the death-inducing signaling complex (DISC) by hindering the binding of the Fas-associated adaptor molecule FADD to the FasR death domain. In addition, it was shown that α2,6-sialylation impaired internalization of the Fas receptor. Normally, internalization of FasR leads to further DISC complex formation and acts as a positive feedback loop for Fas-mediated apoptosis (20). Sialylation of FasR prevented this signal amplification loop and disrupted the downstream apoptotic signaling cascade, allowing tumor cells to disable a major mechanism of apoptosis.

Next to Fas-mediated apoptosis, hypersialylation has been reported to mediate resistance to anoikis, a cell death process triggered when cells detach from adjacent cells or the ECM (21). Amano and colleagues and Sanchez-Ruderisch and colleagues suggested an on/off switch model in which sialylation of the fibronectin receptor α5β1-integrin controls galectin-1–mediated anoikis (22, 23). In this model, galectin-1 binds α5β1-integrin following detachment from fibronectin and triggers proapoptotic signals leading to caspase-8 activation and subsequent cell death. α2,6-hypersialylation of α5β1-integrin prevented its binding to galectin-1 and the subsequent induction of anoikis. These findings support the concept that α2,6−linked sialic acids generally prevent binding of galectins to cell-surface glycans and inhibit galectin signaling (24). Interestingly, in these studies, a correlation was observed between the anoikis-inducing tumor suppressor p16INK4a and expression of genes involved in sialic acid synthesis. p16INK4a selectively upregulates expression of both α5β1-integrin and galectin-1 and reduces α2,6 sialylation by downregulation of two rate-limiting enzymes in sialic acid biosynthesis, UDP-GlcNAc2-epimerase/ManAc kinase (GNE) and sialic acid synthase (NANS; refs. 22, 25). In line with these findings, GNE overexpression has been shown to counteract p16INK4a-induced anoikis, most likely via increasing the metabolic flux through the sialic acid pathway (22, 26).

Sialic Acids and Cancer Progression and Metastasis

Elevated expression of sialoglycans correlates with tumor aggressiveness and their capacity to metastasize and invade surrounding tissue, and therefore correlates with a poor prognosis for patients with cancer (27). Cell biologic aspects of high sialoglycan expression on cell adhesion and motility are well documented and have been extensively reviewed elsewhere (28). Much less is known about the molecular changes during tumor progression that lead to high expression of sialoglycans and a metastatic phenotype. Recent insights reveal that the changes that occur during epithelial–mesenchymal transition (EMT) are associated with altered expression of sialoglycans. EMT is essential for tumor progression and is a prerequisite for cancer cells to invade surrounding tissues and to metastasize. EGF-induced EMT in colon cancer cells resulted in high expression of the sialoglycans sialyl Lewis x (sLex) and sialyl Lewis a (sLea) due to increased expression of ST3Gal I, III, IV (11). sLex and sLea are both ligands for selectins expressed on endothelial cells that allow adherence to blood vessels, facilitate extravasation into surrounding tissue, and trigger angiogenesis. Indeed, high sLea expression correlates with tumor aggressiveness and patient survival (29). In another comprehensive study, Maupin and colleagues analyzed gene expression in a model of TGF-β–induced EMT. Here, EMT induction caused upregulation of the enzymes ST3Gal II, ST6GalNAc IV, and ST8Sia IV, which are involved in the synthesis of the adhesion molecules GD1a and PSA (30). These studies indicate that the upregulation of sialyltransferases and subsequent expression of sialoglycans during EMT represent an important step underlying the migratory phenotype of metastasizing cancer cells.

Further support for the importance of sialic acids in cancer metastasis comes from the scant information that is available about the contribution of sialidases to tumor migration and metastasis. Generally it is believed that downregulation of sialidases in cancer increases their metastatic ability. For example, downregulation of the lysosomal sialidases NEU1 and NEU4 favors tumor metastasis through hypersialylation and enhanced signaling of the laminin receptor β4-integrin or...
Sialic Acids as Targets for Cancer Therapy

In the late 1960s, several groups reported that high expression of sialic acids on tumors favors tumor growth and therefore defined sialic acids as a potential therapeutic target for cancer. Consequently, bacterial sialidases that remove surface sialic acids were utilized for cancer therapy and sialidase-treated tumor cells were used to therapeutically vaccinate patients with cancer in clinical trials, although with limited success (37). For several decades, bacterial sialidases constituted the only robust approach to remove sialic acids from cells, and they are still widely used in research. However, the fact that tumor cells can rapidly replenish sialic acid expression on their cell surface following enzymatic removal severely limits the usage of sialidases for cancer therapy (38).

Today, advances in glycobiology and carbohydrate chemistry have boosted the development of novel strategies to target aberrant sialylation in cancer. Current strategies include specific or global inhibition of sialyltransferases and other enzymes involved in sialic acid biosynthesis (e.g., GNE), overexpression and selective inhibition of sialidases, incorporation of unnatural (antigenic) sialic acid analogues into sialoglycans, and delivery of drugs to tumors using sialic acid-recognizing antibodies or newly developed phenylboronic acid-installed polymeric micelles (39–42). Experimentally, some approaches have already produced promising results in vitro or in tumor mouse models. Our group has recently evaluated the therapeutic potential of a novel sialyltransferase inhibitor, P-3Fax-Neu5Ac, that was developed by Rillahan and colleagues (43). P-3Fax-Neu5Ac is a fluorinated sialic acid analogue that globally inhibits sialyltransferases and prevents the de novo synthesis of sialoglycans with high potency. P-3Fax-Neu5Ac treatment is significantly more effective in prolonged reduction of surface expression of sialic acids on tumor cells when compared with bacterial sialidases. Moreover, in agreement with the proposed consequences of aberrant sialylation for tumor growth, we were able to demonstrate that blockage of sialylation with P-3Fax-Neu5Ac strongly hinders tumor cell adhesion to ECM ligands and migration in vitro and tumor engraftment in a mouse model in vivo (38). Accordingly, Chen and colleagues reported that another sialyltransferase inhibitor, Lith-O-Asp, attenuates spontaneous metastasis formation in a mouse breast cancer model (44). These examples compellingly suggest that the recent approaches to selectively block aberrant sialylation in cancer have the potential to counteract tumor growth and metastasis formation and should be further explored for anticancer therapy.

Conclusions and Future Directions

Sialic acids promote tumorigenesis and enhance tumor progression at multiple levels by facilitating escape from apoptosis, formation of metastasis, and resistance to therapy. Selective approaches interfering with sialic acid expression would therefore affect multiple different key processes in cancer cells simultaneously and hold great promise for cancer treatment. For a long time, only a few compounds interfering with sialic acids were available and data from preclinical trials have been limited. Recently, novel sialyltransferase inhibitors have been developed that show specific and potent blockade of the aberrant sialylation of tumor cells. Moreover, the potential of this new class of inhibitors is emphasized by their ability to antagonize tumor growth and metastasis formation in mouse tumor models. These findings will not only stimulate further basic research into the mechanisms and consequences of aberrant sialylation in cancer, but should also boost further studies on the therapeutic window of opportunities to apply these inhibitors in (pre)clinical models for cancer therapy. It may turn out to be highly rewarding to evaluate the therapeutic potential of these inhibitors for clinical application, either as stand-alone treatment or in combination with other cancer treatment modalities.
Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References
4. Cohen M, Varki A. The sialo–far more than the sum of its parts. OMICS 2010;14:455–64.
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