

Sialic Acids Sweeten a Tumor's Life

Christian Büll, Marieke A. Stoel, Martijn H. den Brok, and Gosse J. Adema

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); 3199–204. ©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 counter parts 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 fifth carbon atom (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 ST6GalNAc I-VI), or to C8 (ST8Sia I-VI) of carbohydrates, yielding α 2,3-, α 2,6-, or α 2,8-linked sialic acids, respectively (3). The latter enzymes are also involved in creating α 2,8-linked polysialic acids (PSA). Together, the multifarious chemical substitu-

tions, 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^{x/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

Authors' Affiliation: Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, the Netherlands

Corresponding Author: Gosse J. Adema, 278 Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Centre P.O. Box 9101, 6500 HB Nijmegen, the Netherlands. Phone: 31-24-36-17600; Fax: 31-24-35-40339; E-mail: g.adema@ncmls.ru.nl

doi: 10.1158/0008-5472.CAN-14-0728

©2014 American Association for Cancer Research.

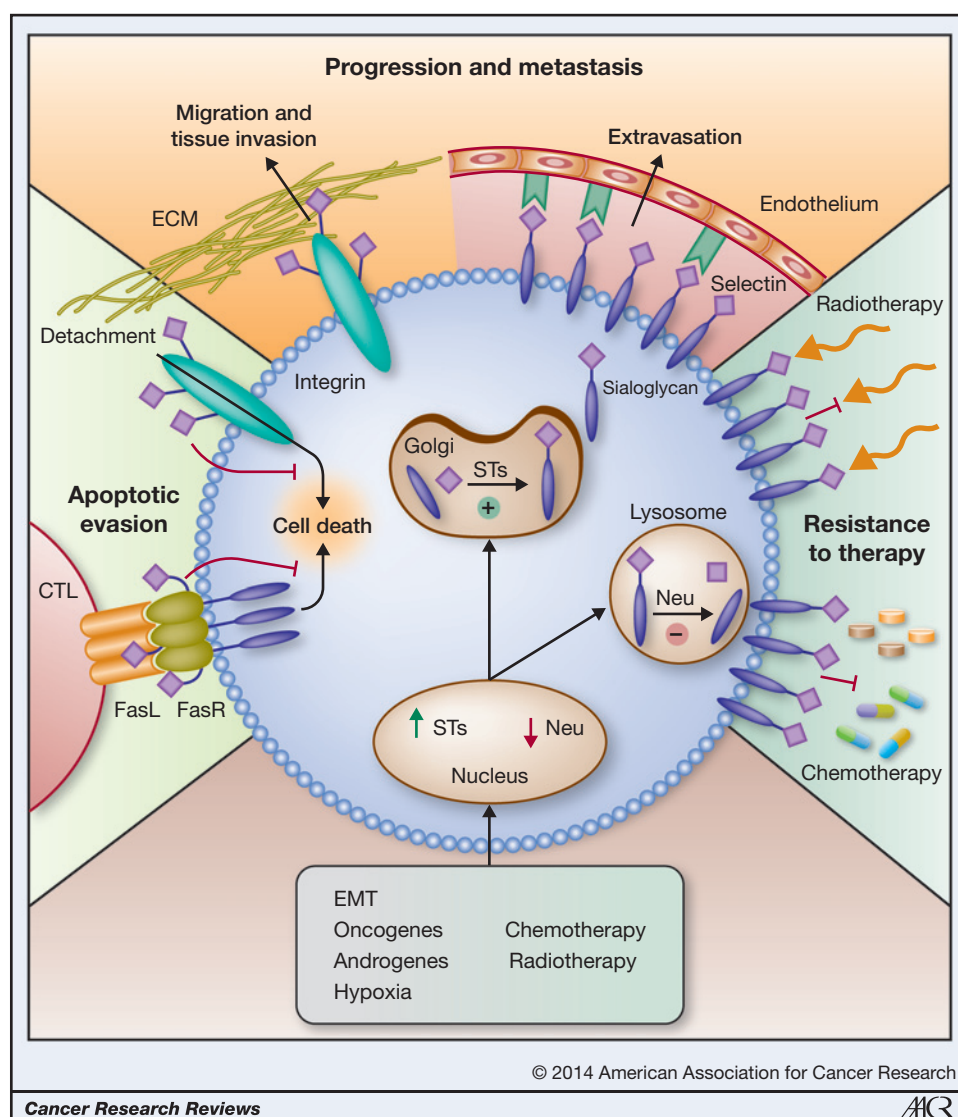


Figure 1. Aberrant sialylation favors tumor growth and progression. Several factors (e.g., oncogenes, hormones, or chemotherapy) increase the expression of sialyltransferases and downregulate the expression of sialidases (Neu) in cancer cells (red). As a result, sialoglycan synthesis in the Golgi system by sialyltransferases is enhanced and hydrolysis of sialoglycans by sialidases in the lysosome is reduced, leading to accumulation of hypersialylated structures on the cell membrane. The aberrant high expression of sialoglycans impairs apoptotic signaling by the Fas receptor or integrins following detachment from the ECM (green); facilitates binding to the ECM or selectins, allowing migration/tissue invasion and metastasis formation, respectively (orange); and mediates resistance to chemo- and radiotherapy (blue). EMT, epithelial-mesenchymal transition.

result in increased $\alpha 2,6$ -sialylation of $\beta 1$ -integrin (Ras) and high expression of sLe^{x/a} antigens (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 sLe^{x/a} 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 GD1a 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.

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 unraveled, 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 tumorigenesis 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 tumorigenesis.

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 α 2,6-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 α 2,6-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 α 5 β 1-integrin controls galectin-1–mediated anoikis (22, 23). In this model, galectin-1 binds α 5 β 1-

integrin following detachment from fibronectin and triggers proapoptotic signals leading to caspase-8 activation and subsequent cell death. α 2,6-hypersialylation of α 5 β 1-integrin prevented its binding to galectin-1 and the subsequent induction of anoikis. These findings support the concept that α 2,6–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 p16^{INK4a} and expression of genes involved in sialic acid synthesis. p16^{INK4a} selectively upregulates expression of both α 5 β 1-integrin and galectin-1 and reduces α 2,6 sialylation by downregulation of two rate-limiting enzymes in sialic acid biosynthesis, UDP-GlcNAc-2-epimerase/ManAc kinase (GNE) and sialic acid synthase (NANS; refs. 22, 25). In line with these findings, GNE overexpression has been shown to counteract p16^{INK4a}-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 (sLe^x) and sialyl Lewis a (sLe^a) due to increased expression of ST3Gal I, III, IV (11). sLe^{x/a} 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 sLe^{x/a} 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 β 4-integrin or

reduced hydrolysis of sLe^x antigens, respectively (31, 32). Additional work is necessary to understand the individual and combined contribution of sialyltransferases and sialidases in cancer cell motility, tissue invasion, and metastasis formation.

Sialic Acids and Resistance to Cancer Therapy

Tumor cell resistance to chemotherapeutics or radiotherapy forms a major barrier toward effective cancer therapy. Recently, several groups provided evidence that aberrant sialylation and especially overexpression of ST6Gal I contribute to therapy resistance in cancer. Schultz and colleagues reported that overexpression of ST6Gal I confers resistance to cisplatin, a platinum-based chemotherapeutic drug frequently used in the clinics. They showed that ST6Gal I knockout sensitizes cancer cells to cisplatin treatment, while overexpression confers resistance. Moreover, they demonstrated that ST6Gal I is highly expressed in cisplatin-resistant cells compared with nonresistant cells (33). However, the effects on α 2,6-sialylation of surface sialoglycans, for example, FasR, need to be determined. Lately, this group also indicated that expression of ST6Gal I regulates cancer stem cell resistance to chemotherapy with irinotecan (17). Importantly, the sialidase NEU3 has recently been found to mediate resistance to the topoisomerase inhibitor etoposide, potentially by affecting surface expression of β 1-integrins and increased FAK/AKT signaling (34).

Other than chemotherapy, Lee and colleagues reported that radiotherapy induces high ST6Gal I expression in both cancer cells and healthy tissue. Radiation of cancer cells increased α 2,6-sialylation of β 1-integrins and could be linked to increased cell adhesion and migration. As is the case with cisplatin resistance, ST6Gal I expression mediated resistance to radiation-induced cell death, but could be reversed upon knockout of ST6Gal I or expression of the plasma sialidase NEU2. Irradiation induced the expression of other sialyltransferases (ST3Gal I-IV, ST8Sia I) as well (35, 36). However, their role in radiation resistance remains to be investigated. Together, the data available so far provide preliminary evidence that altered sialyltransferase and possibly sialidase expression, and hypersialylation of cancer cells can modulate the efficacy of anticancer drugs and confer resistance to chemo- and radiation therapy. These intriguing findings imply that it may be highly rewarding to study the role of sialic acids in cancer therapy resistance further.

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-3F_{ax}-Neu5Ac, that was developed by Rillahan and colleagues (43). P-3F_{ax}-Neu5Ac is a fluorinated sialic acid analogue that globally inhibits sialyltransferases and prevents the *de novo* synthesis of sialoglycans with high potency. P-3F_{ax}-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-3F_{ax}-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.

Acknowledgments

The authors thank Paul Gielen and Mangala Srinivas for carefully reading the manuscript and their useful comments.

Grant Support

This work was supported by a Radboud University Medical Center PhD grant awarded to C. Bill and a grant from the Dutch Cancer Society (KUN2009-4402).

Received March 11, 2014; revised March 27, 2014; accepted March 30, 2014; published OnlineFirst May 15, 2014.

References

- Varki A, Kannagi R, Toole BP. Glycosylation changes in cancer; 2009. In: Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW, Etzler ME editors. *Essentials of Glycobiology*. 2nd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009. Chapter 44.
- Schneider F, Kemmer W, Haensch W, Franke G, Gretschel S, Karsten U, et al. Overexpression of sialyltransferase CMP-sialic acid:Galbeta1,3GalNAc-R alpha6-Sialyltransferase is related to poor patient survival in human colorectal carcinomas. *Cancer Res* 2001;61:4605–11.
- Varki A, Schauer R. Sialic acids. In: Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW, Etzler ME editors. *Essentials of Glycobiology*. 2nd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009. Chapter 14.
- Cohen M, Varki A. The sialome—far more than the sum of its parts. *OMICS* 2010;14:455–64.
- Pillai S, Netravali IA, Cariappa A, Mattoo H. Siglecs and immune regulation. *Annu Rev Immunol* 2012;30:357–92.
- Jandus C, Boligan KF, Chijioko O, Liu H, Dahlhaus M, Demoulin T, et al. Interactions between Siglec-7/9 receptors and ligands influence NK cell-dependent tumor immunosurveillance. *J Clin Invest* 2014;124:1810–20.
- Hudak JE, Canham SM, Bertozzi CR. Glycocalyx engineering reveals a Siglec-based mechanism for NK cell immune evasion. *Nat Chem Biol* 2014;10:69–75.
- Astronomo RD, Burton DR. Carbohydrate vaccines: developing sweet solutions to sticky situations? *Nat Rev Drug Discov* 2010;9:308–24.
- Hauselmann I, Borsig L. Altered Tumor-Cell Glycosylation Promotes Metastasis. *Front Oncol* 2014;4:28.
- Seales EC, Jurado GA, Singhal A, Bellis SL. Ras oncogene directs expression of a differentially sialylated, functionally altered beta1 integrin. *Oncogene* 2003;22:7137–45.
- Sakuma K, Aoki M, Kannagi R. Transcription factors c-Myc and CDX2 mediate E-selectin ligand expression in colon cancer cells undergoing EGF/bFGF-induced epithelial-mesenchymal transition. *Proc Natl Acad Sci U S A* 2012;109:7776–81.
- Koike T, Kimura N, Miyazaki K, Yabuta T, Kumamoto K, Takenoshita S, et al. Hypoxia induces adhesion molecules on cancer cells: A missing link between Warburg effect and induction of selectin-ligand carbohydrates. *Proc Natl Acad Sci U S A* 2004;101:8132–7.
- Hatano K, Miyamoto Y, Mori M, Nimura K, Nakai Y, Nonomura N, et al. Androgen-regulated transcriptional control of sialyltransferases in prostate cancer cells. *PLoS ONE* 2012;7:e31234.
- Almaraz RT, Tian Y, Bhattacharya R, Tan E, Chen SH, Dallas MR, et al. Metabolic flux increases glycoprotein sialylation: implications for cell adhesion and cancer metastasis. *Mol Cell Proteomics* 2012;11:M112017558.
- Miyagi T, Takahashi K, Hata K, Shiozaki K, Yamaguchi K. Sialidase significance for cancer progression. *Glycoconj J* 2012;29:567–77.
- Picco G, Julien S, Brockhausen I, Beatson R, Antonopoulos A, Haslam S, et al. Over-expression of ST3Gal-I promotes mammary tumorigenesis. *Glycobiology* 2010;20:1241–50.
- Swindall AF, Londono-Joshi AI, Schultz MJ, Fineberg N, Buchsbaum DJ, Bellis SL. ST6Gal-I protein expression is upregulated in human epithelial tumors and correlates with stem cell markers in normal tissues and colon cancer cell lines. *Cancer Res* 2013;73:2368–78.
- Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646–74.
- Swindall AF, Bellis SL. Sialylation of the Fas death receptor by ST6Gal-I provides protection against Fas-mediated apoptosis in colon carcinoma cells. *J Biol Chem* 2011;286:22982–90.
- Lee KH, Feig C, Tchikov V, Schickel R, Hallas C, Schutze S, et al. The role of receptor internalization in CD95 signaling. *EMBO J* 2006;25:1009–23.
- Guadamillas MC, Cerezo A, Del Pozo MA. Overcoming anoikis—pathways to anchorage-independent growth in cancer. *J Cell Sci* 2011;124:3189–97.
- Amano M, Eriksson H, Manning JC, Detjen KM, Andre S, Nishimura S, et al. Tumour suppressor p16(INK4a) - anoikis-favouring decrease in N/O-glycan/cell surface sialylation by down-regulation of enzymes in sialic acid biosynthesis in tandem in a pancreatic carcinoma model. *FEBS J* 2012;279:4062–80.
- Sanchez-Ruderisch H, Detjen KM, Welzel M, Andre S, Fischer C, Gabius HJ, et al. Galectin-1 sensitizes carcinoma cells to anoikis via the fibronectin receptor alpha5beta1-integrin. *Cell Death Differ* 2011;18:806–16.
- Zhuo Y, Bellis SL. Emerging role of alpha2,6-sialic acid as a negative regulator of galectin binding and function. *J Biol Chem* 2011;286:5935–41.
- Plath T, Detjen K, Welzel M, von Marschall Z, Murphy D, Schirner M, et al. A novel function for the tumor suppressor p16(INK4a): induction of anoikis via upregulation of the alpha(5)beta(1) fibronectin receptor. *J Cell Biol* 2000;150:1467–78.
- Kemmer W, Kessel P, Sanchez-Ruderisch H, Moller H, Hinderlich S, Schlag PM, et al. Loss of UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE) induces apoptotic processes in pancreatic carcinoma cells. *FASEB J* 2012;26:938–46.
- Julien S, Ivetic A, Grigoriadis A, QiZe D, Burford B, Sproviero D, et al. Selectin ligand sialyl-Lewis x antigen drives metastasis of hormone-dependent breast cancers. *Cancer Res* 2011;71:7683–93.
- Schultz MJ, Swindall AF, Bellis SL. Regulation of the metastatic cell phenotype by sialylated glycans. *Cancer Metastasis Rev* 2012;31:501–18.
- Nakamori S, Kameyama M, Imaoka S, Furukawa H, Ishikawa O, Sasaki Y, et al. Increased expression of sialyl Lewis x antigen correlates with poor survival in patients with colorectal carcinoma: clinicopathological and immunohistochemical study. *Cancer Res* 1993;53:3632–7.
- Maupin KA, Sinha A, Eugster E, Miller J, Ross J, Paulino V, et al. Glycogene expression alterations associated with pancreatic cancer epithelial-mesenchymal transition in complementary model systems. *PLoS One* 2010;5:e13002.
- Uemura T, Shiozaki K, Yamaguchi K, Miyazaki S, Satomi S, Kato K, et al. Contribution of sialidase NEU1 to suppression of metastasis of human colon cancer cells through desialylation of integrin beta4. *Oncogene* 2009;28:1218–29.
- Shiozaki K, Yamaguchi K, Takahashi K, Moriya S, Miyagi T. Regulation of sialyl Lewis antigen expression in colon cancer cells by sialidase NEU4. *J Biol Chem* 2011;286:21052–61.
- Schultz MJ, Swindall AF, Wright JW, Sztul ES, Landen CN, Bellis SL. ST6Gal-I sialyltransferase confers cisplatin resistance in ovarian tumor cells. *J Ovarian Res* 2013;6:25.
- Tringali C, Lupo B, Silvestri I, Papini N, Anastasia L, Tettamanti G, et al. The plasma membrane sialidase NEU3 regulates the malignancy of renal carcinoma cells by controlling beta1 integrin internalization and recycling. *J Biol Chem* 2012;287:42835–45.
- Lee M, Lee HJ, Bae S, Lee YS. Protein sialylation by sialyltransferase involves radiation resistance. *Mol Cancer Res* 2008;6:1316–25.

36. Lee M, Lee HJ, Seo WD, Park KH, Lee YS. Sialylation of integrin beta1 is involved in radiation-induced adhesion and migration in human colon cancer cells. *Int J Radiat Oncol Biol Phys* 2010;76:1528–36.
37. Sedlacek HH, Seiler FR. Immunotherapy of neoplastic diseases with neuraminidase - contradictions, new aspects, and revised concepts. *Cancer Immunol Immun* 1978;5:153–63.
38. Büll C, Boltje TJ, Wassink M, de Graaf AM, van Delft FL, den Brok MH, et al. Targeting aberrant sialylation in cancer cells using a fluorinated sialic acid analogue impairs adhesion, migration and in vivo tumor growth. *Mol Cancer Ther* 2013;12:1935–46.
39. Deshayes S, Cabral H, Ishii T, Miura Y, Kobayashi S, Yamashita T, et al. Phenylboronic Acid-installed polymeric micelles for targeting sialylated epitopes in solid tumors. *J Am Chem Soc* 2013;135:15501–7.
40. Fuster MM, Esko JD. The sweet and sour of cancer: glycans as novel therapeutic targets. *Nat Rev Cancer* 2005;5:526–42.
41. Ochoa-Alvarez JA, Krishnan H, Shen YQ, Acharya NK, Han M, McNulty DE, et al. Plant lectin can target receptors containing sialic acid, exemplified by podoplanin, to inhibit transformed cell growth and migration. *PLoS ONE* 2012;7:e41845.
42. O'Shea LK, Abdulkhalek S, Allison S, Neufeld RJ, Szewczuk MR. Therapeutic targeting of Neu1 sialidase with oseltamivir phosphate (Tamiflu(R)) disables cancer cell survival in human pancreatic cancer with acquired chemoresistance. *Onco Targets Ther* 2014;7:117–34.
43. Rillahan CD, Antonopoulos A, Lefort CT, Sonon R, Azadi P, Ley K, et al. Global metabolic inhibitors of sialyl- and fucosyltransferases remodel the glycome. *Nat Chem Biol* 2012;8:661–8.
44. Chen JY, Tang YA, Huang SM, Juan HF, Wu LW, Sun YC, et al. A novel sialyltransferase inhibitor suppresses FAK/paxillin signaling and cancer angiogenesis and metastasis pathways. *Cancer Res* 2011;71:473–83.