The Glycosyltransferase ST6Gal-I Protects Tumor Cells against Serum Growth Factor Withdrawal by Enhancing Survival Signaling and Proliferative Potential*

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A hallmark of cancer cells is the ability to survive and proliferate when challenged with stressors such as growth factor insufficiency. In this study, we report a novel glycosylation-dependent mechanism that protects tumor cells from serum growth factor withdrawal. Our results suggest that the β-galactoside α-2,6-sialyltransferase 1 (ST6Gal-I) sialyltransferase, which is up-regulated in numerous cancers, promotes the survival of serum-starved cells. Using ovarian and pancreatic cancer cell models with ST6Gal-I overexpression or knockdown, we find that serum-starved cells with high ST6Gal-I levels exhibit increased activation of prosurvival signaling molecules, including pAkt, p-p70S6K, and pNFκB. Correspondingly, ST6Gal-I activity augments the expression of tumor-promoting pNFκB transcriptional targets such as IL-6, IL-8, and the apoptosis inhibitor clAP2. ST6Gal-I also potentiates expression of the cell cycle regulator cyclin D2, leading to increased phosphorylation and inactivation of the cell cycle inhibitor pRB. Consistent with these results, serum-starved cells with high ST6Gal-I expression maintain a greater number of S phase cells compared with low ST6Gal-I expressors, reflecting enhanced proliferation. Finally, selective enrichment in clonal variants with high ST6Gal-I expression is observed upon prolonged serum deprivation, supporting the concept that ST6Gal-I confers a survival advantage. Collectively, these results implicate a functional role for ST6Gal-I in fostering tumor cell survival within the serum-depleted tumor microenvironment.

Cell surface glycans lie at the interface between the extracellular milieu and intracellular signaling networks that regulate cellular response to the microenvironment. Glycosylation of surface receptors can have profound effects on cell signaling through glycan-dependent modulation of receptor conformation, clustering, surface retention, and/or interaction with other membrane proteins. The profile of glycans on membrane receptors changes dramatically in correspondence with numerous pathophysiologic conditions, including malignant transformation (1–4). However, the functional role of aberrant glycosylation as a transducer of tumor microenvironmental cues remains poorly understood.

One important glycosyltransferase dysregulated in cancer cells is the ST6Gal-I2-6 sialyltransferase (5–8). ST6Gal-I adds α2-6-linked sialic acids to N-glycans on select receptors, including the β1 integrin receptor (9–11), the Fas and TNFR1 death receptors (12, 13), the growth factor receptor EGFR receptor (EGFR) (14), and multiple other membrane proteins. ST6Gal-I expression is markedly up-regulated in numerous malignancies, including colon, pancreatic, and ovarian adenocarcinoma (15–17). In ovarian cancer, high levels of ST6Gal-I correlate with reduced progression-free and overall patient survival, and ST6Gal-I expression is enriched in metastatic versus primary ovarian tumors (16). Contrarily, ST6Gal-I expression is negligible in the differentiated epithelium of normal colon, pancreas, and ovary (15, 16), whereas, notably, a subset of cells with high ST6Gal-I expression is found within the base of colon crypts, a stem cell niche (15). ST6Gal-I is also highly expressed in other epithelium-related stem cell compartments (15, 16) as well as in embryonic and induced pluripotent stem cells (15, 18–20).

Consistent with a potential function in conferring stem cell-like properties, we recently reported that ST6Gal-I promotes a cancer stem cell (CSC) phenotype (16). ST6Gal-I expression correlates with other CSC markers, including ALDH1 and CD133 (15), and ST6Gal-I activity is critical for CSC behaviors such as tumor spheroid growth, chemoresistance and tumorigenesis initiating potential (16). Additionally, ST6Gal-I up-regulation in tumor cells induces the expression of important CSC-associated transcription factors, including Sox9 and Slug (16). We hypothesize that one of the main functions of ST6Gal-I in cancer cells, including CSCs, is to protect against diverse cytotoxic stimuli through sialylation-dependent modulation of cell surface receptors. For example, α2-6 sialylation of the Fas receptor inhibits apoptosis by preventing Fas internalization, a requisite step in apoptotic signaling (12). Furthermore, α2-6 sialylation

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2 The abbreviations used are: ST6Gal-I, β-galactoside α-2,6-sialyltransferase 1; EGFR, EGF receptor; CSC, cancer stem cell; OE, overexpression; EV, empty vector; KD, knockdown; AIF, apoptosis-inducing factor; RB, retinoblastoma protein; qRT-PCR, quantitative RT-PCR; SNA, Sambucus nigra agglutinin; MAA, Maackia amurensis agglutinin; EdU, 5-ethynyl-2’-deoxyuridine.
of TNFR1 blocks TNFα-stimulated cell death (13), whereas α2-6 sialylation of certain integrins impedes apoptosis induced by galectins (21, 22), a family of galactose-binding lectins. In immune cells, ST6Gal-I-mediated sialylation of CD45 prevents galectin-dependent CD45 clustering and subsequent apoptosis (23). Finally, α2-6 sialylation of the platelet endothelial cell adhesion molecule receptor prevents endothelial cell death (24).

In this study, we describe a new survival-associated function for ST6Gal-I: protection against serum growth factor insufficiency. Using ovarian and pancreatic cancer cell models with ST6Gal-I overexpression or knockdown, we show that ST6Gal-I facilitates the survival of cells grown under serum-depleted culture conditions. Serum-starved cells with high ST6Gal-I expression maintain activation of prosurvival signaling nodes, including Akt and NFκB, and retain proliferative capacity via ST6Gal-I-dependent up-regulation of cyclin D2. These findings highlight a novel role for the tumor glycome in sustaining the viability of tumor cells exposed to serum-depleted environments, such as those found within hypovascularized regions of large, solid tumors.

**Results**

*Cells with High ST6Gal-I Expression Are Resistant to Cytotoxic Stress Induced by Serum Deprivation—* To interrogate the role of ST6Gal-I in protection against serum withdrawal, ST6Gal-I was stably overexpressed in the OV4 ovarian cancer cell line, which is one of the few cancer lines that lack detectable ST6Gal-I protein (Fig. 1A). As expected, overexpression (OE) of ST6Gal-I led to a pronounced increase in surface α2-6 sialylation relative to empty vector (EV) control cells, as measured by the binding of SNA (Fig. 1B). The SNA lectin specifically detects α2-6 sialic acids. Contrarily, ST6Gal-I overexpression had no effect on α2-3 sialylation, evidenced by equivalent binding of the MAA lectin to EV and OE cells (Fig. 1C).

OV4 EV or OE cells were grown in medium containing 10%, 1%, or 0% FBS for 1 week. As shown in Fig. 1D, EV cells grown in 1% FBS displayed a spindle-shaped morphology, reflecting cell stress, whereas EV cells grown in 0% FBS exhibited rounding and detachment from the plate, indicative of cell death. In contrast to EV cells, OE cells maintained a spread morphology and formed a confluent monolayer, even in 0% FBS, suggesting that ST6Gal-I promotes cell survival.

In complementary studies, the effect of ST6Gal-I knockdown was examined. BxPC3 pancreatic cancer cells, which have moderate levels of endogenous ST6Gal-I, were stably transduced with a lentivirus encoding ST6Gal-I, and ST6Gal-I OE was confirmed by immunoblotting. Control cells were generated by stable transduction of an EV lentiviral construct (Fig. 1E). BxPC3 EV and OE cells were stained with SNA-FITC and evaluated by flow cytometry. C, OV4 EV and OE cells were stained with MAA-FITC and evaluated by flow cytometry. D, OV4 EV and OE cells were cultured in 10%, 1%, or 0% FBS-containing medium for 1 week and imaged to observe differences in cell morphology. E, BxPC3 cells were stably transduced with shRNA for ST6Gal-I using a lentivirus, and ST6Gal-I KD was verified by immunoblotting. F, BxPC3 cells were stained with SNA-FITC and evaluated by flow cytometry. G, BxPC3 cells were stained with MAA-FITC and evaluated by flow cytometry. H, EV and KD BxPC3 cells were cultured in 10% or 1% FBS-containing medium for 1 week and then imaged.

To further probe the role of ST6Gal-I in protection against serum withdrawal, OV4 and BxPC3 cells were grown for 1 week in 10% or 1% FBS, lysed, and immunoblotted for survival-associated signaling molecules. Under conditions of serum-depletion, OV4 OE cells expressed higher levels of activated (phosphorylated) Akt as well as the apoptosis inhibitor cIAP2 relative to EV cells (representative blots in Fig. 2A and densitometric analyses of three independent blots in Fig. 2B). Interestingly, pAkt and cIAP2 expression was also higher in the OE versus EV cultures grown in 10% FBS, suggesting that ST6Gal-I enhances basal activation of these molecules. Consistent with these results, serum-starved BxPC3 cells with ST6Gal-I knockdown had reduced levels of pAkt and cIAP2 compared with EV cells (representative blots in Fig. 2C and densitometry in Fig. 2D). We also immunoblotted for apoptosis-inducing factor (AIF), which is a caspase-independent cell death marker associated with the intrinsic apoptotic pathway. OV4 OE cells had lower expression of AIF than EV cells under conditions of both 10% and 1%
FBS. However, unlike OV4 cells, AIF expression in BxPC3 cells was equivalent in the serum-starved EV and KD lines. Additionally, we evaluated Beclin expression, a marker for autophagy. No significant correlations were noted between Beclin levels and ST6Gal-I activity in the two cell models, suggesting that autophagy may not be a major factor in the protective effect of ST6Gal-I, although further studies are clearly needed.

Other studies from our group have suggested that cytotoxic stimuli can exert selective pressure, leading to the expansion of clonal variants with high ST6Gal-I expression. For example, cell lines developed to grow continuously in the presence of the chemotherapeutics cisplatin or irinotecan have up-regulated ST6Gal-I (15, 25). Accordingly, we investigated whether prolonged exposure to serum-depleted culture conditions would select for cells with high ST6Gal-I expression. OV4 EV and KD cells were serum-deprived for 1 week and immunoblotted for survival or cell death markers as in A and C. BxPC3 EV and KD cells were serum-deprived for 1 week and immunoblotted for survival or cell death markers as in A and D. Similar results were noted for BxPC3 cells (Fig. 3, C and D); robust ST6Gal-I up-regulation was apparent in EV cells grown in 1% versus 10% serum.

**ST6Gal-I Protects against Serum Deprivation**

**FIGURE 2. ST6Gal-I enhances survival signaling in serum-starved cells.** A, OV4 cells were serum deprived for 1 week and then immunoblotted for survival (pAkt and clAP2) or cell death (Beclin and AIF) markers. Representative blots are shown. B, densitometric analyses of p-Akt, total Akt, clAP2, AIF, and Beclin. Three distinct blots (from three independently generated cell lysates) were evaluated using ImageJ, and data were plotted as mean ± S.D. *, p < 0.05. For Akt, pAkt and total Akt were each normalized to β-tubulin, and then data were plotted as pAkt/total Akt (p/t Akt). For clAP2, AIF, and Beclin, values were normalized to β-tubulin. C, BxPC3 EV and KD cells were serum-deprived for 1 week and immunoblotted for survival or cell death markers as in A. D, densitometric analyses of three independent blots for pAkt, total Akt, clAP2, AIF, and Beclin. Data were normalized to β-tubulin as described in B. *, p < 0.05.
shown in Fig. 4, A and B, OV4 OE cells displayed higher levels of pAkt than EV cells at all time points under 1% FBS culture conditions. Of note, there was a substantial decrease in pAkt in EV cells exposed to 1% FBS relative to 10% FBS, consistent with repression of survival signaling networks. We also evaluated phosphorylation of p70S6K, a kinase downstream of Akt that plays an important role in protein synthesis (26). The transla-

tory of p70S6K, which may reflect a compensatory survival response. ST6Gal-I also regulated cyclin D2 expression in BxPC3 cells (Fig. 7B). KD cells had reduced levels of cyclin D2 mRNA relative to EV cells regardless of FBS concentration. These results were validated by immunoblotting, which showed striking differences in cyclin D2 protein expression in cells with differential ST6Gal-I activity. OV4 OE cells had substantially greater levels of cyclin D2 than EV cells, which were further enriched in OE cells grown in 1% FBS (Fig. 7, C and E). Similarly, BxPC3 EV cells had greater levels of cyclin D2 than KD cells (Fig. 7, D and F).

Cyclin D2 partners with select cyclin-dependent kinases to mediate phosphorylation of the retinoblastoma protein (pRb), a molecule that represses transition into S phase (29). Phosphorylation of pRb leads to pRb degradation, thus allowing the cell cycle to proceed. Serum-starved OV4 OE cells exhibited increased levels of both phosphorylated and total pRb compared with EV cells (Fig. 7, G and I), whereas serum-starved BxPC3 KD cells had diminished p-pRb and total pRb relative to EV cells (Fig. 7, H and J). These results suggest that ST6Gal-I activity promotes cyclin D2 expression and pRb phosphorylation, which would be expected to facilitate entry into S phase.

**Cells with High ST6Gal-I Expression Maintain a Greater Number of Cells in S Phase following Serum Deprivation**—To determine the role of ST6Gal-I in regulating cell cycle progression, we quantified the percentage of cells in S phase. To this end, cells were incubated with EdU, a thymidine analog that incorporates into the DNA during DNA replication. EdU incorporation was monitored by flow cytometry. Upon serum deprivation, OV4 OE cells maintained a significantly greater percentage of S phase cells than the EV line. On the other hand, the number of S phase cells was equivalent for EV and OE cells grown in 10% FBS (a representative experiment is shown in Fig. 8A; an average of three independent experiments is shown in Fig. 8B). BxPC3 cells recapitulated this phenotype. Serum-starved BxPC3 EV cells had a greater abundance of S phase cells than serum-starved KD cells, whereas no differences were noted in the 10% FBS cultures (Fig. 8, C and D). These data suggest that ST6Gal-I does not significantly affect cell proliferation when serum growth factors are replete but, instead, acts to maintain cell proliferative capacity when growth factors are withdrawn.
Discussion

The ability to survive and proliferate in the absence of growth factors is a defining feature of a tumor cell. Dysregulation of growth factor signaling networks comprises one of the principal characteristics of a transformed cell (30). One important mechanism tumor cells utilize to overcome growth factor deprivation is to up-regulate the expression of growth factors or growth factor receptors (31). Alternatively, cancer cells acquire activating mutations in growth factor receptors or downstream effector molecules, including ras, raf, and PI3K (30, 32). In addition to these established pathways, this study elucidates a novel glycosylation-dependent mechanism that protects tumor cells from the cytotoxic stress exerted by a growth factor-deficient environment.

More specifically, we show that the sialyltransferase ST6Gal-I is critical for maintaining prosurvival signaling cascades in cells exposed to serum starvation. Forced overexpression of ST6Gal-I enhances signaling through survival-associated molecules, including pAkt, p-p70S6K, pNFκB, and cIAP2, whereas ST6Gal-I knockdown inhibits these same proteins. Akt is a key downstream mediator of growth factor receptor activity, and this kinase plays a seminal role in protecting cells from a variety of stressors, including serum withdrawal (33–35). A positive association between ST6Gal-I and Akt activation has been reported previously, for example, during epithelial-to-mesenchymal transition, a process shown to be dependent on ST6Gal-I (36). As with Akt, the NFκB signaling axis is essential for cell survival (37), and, interestingly, Akt...
cross-talks with NFκB to confer protection against apoptosis (38, 39). NFκB directs the transcription of numerous molecules important for cell survival and proliferation (cIAP2), angiogenesis (IL-8), and inflammation (IL-6) (40). Our studies show that ST6Gal-I potentiates NFκB activation, leading to increased expression of cIAP2, IL-8, and IL-6. Of particular significance, cIAP2 is a potent inhibitor of apoptosis.

ST6Gal-I activity also promotes the expression of another major NFκB target, cyclin D2, a cell cycle gateway molecule that controls the transition between G1 and S phase. Cyclin D2 binds and activates cyclin-dependent kinases 4 and 6 (cdk4 and cdk6), and this complex then directs phosphorylation of pRb (29). pRb phosphorylation represses its normal function, which is to block progression into S phase. In accordance with ST6Gal-I-induced expression of cyclin D2, cells with high ST6Gal-I levels display increased phosphorylation of pRb and a corresponding enrichment in the proportion of cells that have entered S phase. These collective results point to a role for ST6Gal-I in enabling tumor cells to sustain proliferative capacity when challenged by a serum-depleted microenvironment. This concept is further supported by our finding that, after a 1-week exposure to serum deprivation, ST6Gal-I levels are elevated in the surviving population. We hypothesize that this reflects the selective survival and expansion of clonal variants with high ST6Gal-I expression.

The observation that ST6Gal-I protects cells against serum withdrawal adds to the growing body of literature suggesting that ST6Gal-I serves as a crucial tumor cell survival factor. ST6Gal-I expression is induced by oncogenes such as ras (41, 42) as well as the stem cell-associated transcription factor Sox2.
Up-regulation of ST6Gal-I in cancer cells functions to confer resistance to many antitumor treatments, including radiation (44) and the chemotherapeutic agents cisplatin (25), gemcitabine (16), and docetaxel (45). One likely mechanism by which ST6Gal-I fosters tumor cell survival is through imparting a CSC-like phenotype. CSCs are notoriously resistant to a multiplicity of death-inducing stimuli, including radiation, chemotherapy, and hypoxia (46). Relevant to this investigation, extensive literature has established that CSCs are better able to survive and proliferate under conditions of serum withdrawal than more differentiated tumor cells (47, 48). Reciprocally, long-term culture of cancer cells in serum-deficient medium is used as a method to isolate CSCs from a heterogeneous cell population (47).

Further studies will be needed to define the surface receptors that mediate ST6Gal-I-dependent resistance to serum deprivation. There are some established ST6Gal-I substrates that play fundamental roles in cell survival, including members of the integrin, death receptor, and growth factor receptor families. As an example, EGFR is known to be \(\alpha 2-6\)-sialylated, although,
In this instance, sialylation appears to inhibit EGFR activation (14, 49). However, rather than a single, linear signaling pathway, it is possible that multiple differentially sialylated receptors act in concert to induce intracellular signaling events that confer the anti-apoptotic properties associated with CSCs. In essence, ST6Gal-I may function in an oncogene-like manner to coordinately regulate a select cohort of tumor cell receptors. The concept that certain glycosyltransferases could have an oncogene-like function has received limited attention despite the fact that an altered surface glycome was one of the earliest identified markers of a tumor cell. The results presented in this study provide an important advance by highlighting a new function for ST6Gal-I in promoting the viability of tumor cells exposed to the type of serum-depleted conditions often found in hypovascularized tumor microenvironments.

**Experimental Procedures**

**Cell Culture**—For routine propagation of cell lines, cells were grown in DMEM/F12 (OV4) or RPMI (BxPC3) medium containing 10% FBS and 1% antibiotic/antimycotic supplements. The antibiotic/antimycotic stock solution was purchased from Invitrogen and diluted 1:100 into medium. For studies of serum deprivation, cells were cultured for the indicated times using medium supplemented with either 10%, 1%, or 0% FBS. Stable polyclonal cell lines were created by transducing cells with a lentivirus encoding either the ST6Gal-I gene (Genecopoeia) or a control gene.
shRNA against ST6Gal-I (Sigma, TRCN00000035432, sequence CCGGCGTGTGCTACTACTACCAGAACTCGAGTTCTG-GTAGTAGTAGACACGTTTTTG), followed by selection with puromycin. ST6Gal-I overexpression or knockdown was verified by immunoblotting whole cell lysates with anti-ST6Gal-I goat polyclonal antibody (R&D Systems, AF5924) and by quantitative real-time PCR (Applied Biosystems). In addition, we conducted lectin staining to confirm differential ST6Gal-I activity. Cells were stained with either FITC-conjugated SNA lectin (EY Laboratories, F-6802-1), which is specific for α2-6 sialic acids, or FITC-MAA (EY Laboratories, F-7801-2), which binds to α2-3 sialic acids. Cells were stained for 40 min at 4 °C with a 1:200 dilution of SNA-FITC or a 1:80 dilution of MAA-FITC, and then binding of the lectin was quantified by flow cytometry.

Immunoblotting—Cells were serum-deprived for the indicated times and then lysed using radioimmune precipitation assay buffer supplemented with protease and phosphatase inhibitors (Sigma). Total protein concentration was measured by BCA (Pierce). Samples were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Membranes were incubated with 5% nonfat dry milk in TBS contain-

FIGURE 8. ST6Gal-I activity promotes cell cycle progression in serum-starved cells. To assay for cell cycle progression, cells in S phase were labeled with the Click-it EdU reagent (Thermo) and then quantified by flow cytometry. A, representative experiment showing a greater percentage of S phase cells in serum-starved OV OE compared with EV cells. B, three independent flow cytometry experiments measuring the percentage of cells in S phase. *, p < 0.05. C, representative experiment showing a greater percentage of S phase cells in serum-starved BxPC3 EV versus KD cells. D, three independent flow cytometry experiments measuring the percentage of cells in S phase. *, p < 0.05.
ing 0.1% Tween 20 (TBST). Immunoblots were probed with antibodies to ST6Gal-I (R&D Systems, AF5924), cIAP2 (Cell Signaling Technology, 3130, lot 6), pAkt (Ser-473, Cell Signaling Technology, 4060, lot 19), total Akt (Cell Signaling Technology, 4691, lot 20), ALF (Cell Signaling Technology, 4642, lot 3), Beclin (Cell Signaling Technology, 3495, lot 2), p-p70S6K (Thr-389, Cell Signaling Technology, 9234, lot 11), total p70S6K (Cell Signaling Technology, 3708, lot 4), cyclin D2 (Cell Signaling Technology, 3741, lot 4), p-pRB (Ser-809/811, Cell Signaling Technology, 8516, lot 4), or total pRb (Cell Signaling Technology, 3741, lot 4), p-p70S6K (Ser-536, Cell Signaling Technology, 3033, lot 14), total NFκB (p65, Cell Signaling Technology, 8516, lot 4), or total Beclin (Cell Signaling Technology, 3495, lot 2). Protein loading was verified using anti-β-actin (Abcam, ab20272) or anti-β-tubulin (Abcam, ab21058). Membranes were incubated with horseradish peroxidase-coupled secondary antibodies (Cell Signaling Technology, 7074). Immunoblotting for all targets was performed with ImageJ software. All bands were normalized to their respective β-tubulin loading controls. Student’s t test was employed to determine significance (p < 0.05).

qRT-PCR—RNA was extracted using the protocol for the RNeasy Plus mini kit (Qiagen). Total RNA concentration was measured, and cDNA was made using the SuperScript VILO cDNA synthesis kit (Thermo). qRT-PCR samples were prepared using TaqMan Fast Advanced Master Mix (Thermo). Primers for cIAP2 (Hs00985031_g1), IL-6 (Hs00171431_m1), IL-8 (Hs00174103_m1), and cyclin D2 (Hs00153380_m1) were acquired from Applied Biosysytems. Data were normalized to GAPDH (Applied Biosystems, Hs00266249_g1) and significance was determined as p < 0.05 using Student’s t test from at least four independent experiments, with each independent experiment performed in triplicate.

Flow Cytometry and Cell Cycle Analysis—S phase cells were measured using flow cytometry by following the protocol accompanying the Click-It Edu Alexa Fluor 488 flow cytometry assay kit (Thermo). Cells were incubated with 7.5 μM Edu for 2 h prior to cell labeling and analysis. For the Edu assays, OV4 cells were incubated in 0% FBS for 96 h, whereas BxPC3 cells were incubated in 1% serum for 24 h. These conditions were adopted because OV4 cells appear to have greater inherent resistance to serum deprivation.

Author Contributions—C. M. B. was responsible for the acquisition and analysis of the data, with oversight from S. L. B. K. A. D. aided with the collection and analysis of flow cytometry data. C. M. B. and S. L. B. were responsible for the concept and design of this study and together wrote the manuscript.

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References
1. Munkley, J., and Elliott, D. J. (2016) Hallmarks of glycosylation in cancer. Oncotarget 7, 35478–35489
2. Takahashi, M., Kizuoka, Y., Ohtsuka, K., Gu, J., and Taniguchi, N. (2016) Disease-associated glycans on cell surface proteins. Mol. Aspects Med. 51, 56–70.
3. Stowell, S. R., Ju, T., and Cummings, R. D. (2015) Protein glycosylation in cancer. Annu. Rev. Pathol. 10, 473–510
4. Pinho, S. S., and Reis, C. A. (2015) Glycosylation in cancer: mechanisms and clinical implications. Nat. Rev. Cancer 15, 540–555
5. Lu, J., and Gu, J. (2015) Significance of β-galactoside α2,6 sialyltransferase 1 in cancers. Molecules 20, 7509–7527
6. Schultz, M. J., Swindall, A. F., and Bellis, S. L. (2012) Regulation of the metastatic cell phenotype by sialylated glycans. Cancer Metastasis Rev. 31, 501–518
7. Büll, C., Stoe1, M. A., den Brok, M. H., and Adema, G. J. (2014) Sialic acids sweeten a tumor’s life. Cancer Res. 74, 3199–3204
8. Dall’Olio, F., Malagolini, N., Trinchera, M., and Chiricolo, M. (2014) Sialosignaling: sialyltransferases as engines of self-fuelling loops in cancer progression. Biochim. Biophys. Acta 1840, 2752–2764
9. Seales, E. C., Jurado, G. A., Brunson, B. A., Wakefield, J. K., Frost, A. R., and Bellis, S. L. (2005) Hypersialylation of β1 integrins, observed in colon adenocarcinoma, may contribute to cancer progression by up-regulating cell motility. Cancer Res. 65, 4645–4652
10. Hou, S., Hang, Q., Isaji, T., Lu, J., Fukuda, T., and Gu, J. (2016) Importance of membrane-proximal N-glycosylation on integrin β1 in its activation and complex formation. FASEB J. 30, 4120–4131
11. Ip, C. K., Yung, S., Chan, T. M., Tsao, S. W., and Wong, A. S. (2014) p70S6 kinase drives ovarian cancer metastasis through multicellular spheroid-peritoneum interaction and P-cadherin/β1 integrin signaling activation. Oncotarget 5, 9133–9149
12. Swindall, A. F., and Bellis, S. L. (2011) Sialylation of the Fas death receptor by ST6Gal-I provides protection against Fas-mediated apoptosis in colon carcinoma cells. J. Biol. Chem. 286, 22982–22990
13. Liu, Z., Swindall, A. F., Kesterson, R. A., Schoeb, T. R., Bullard, D. C., and Bellis, S. L. (2011) ST6Gal-I regulates macrophage apoptosis via α2-6 sialylation of the TNFR1 death receptor. J. Biol. Chem. 286, 39654–39662
14. Park, J. J., Yi, J. Y., Jin, Y. B., Lee, Y. J., Lee, S. J., Lee, Y. S., Ko, Y. G., and Lee, M. (2012) Sialylation of epidermal growth factor receptor regulates receptor activity and chemosensitivity to gefitinib in colon cancer cells. Biochim. Biophys. Acta 83, 849–857
15. Swindall, A. F., Londoño-Joshi, A. I., Schultz, M. J., Fineberg, N., Buchsbaum, D. J., and Bellis, S. L. (2013) 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. 73, 2368–2378
16. Schultz, M. J., Holdbrooks, A. T., Chakrabarty, A., Grizzle, W. E., Landen, C. N., Buchsbaum, D. J., Conner, M. G., Arend, R. C., Yoon, K. J., Klug, C. A., Bullard, D. C., Kesterson, R. A., Oliver, P. G., O’Connor, A. K., Yoder, B. K., and Bellis, S. L. (2016) The tumor-associated glycosyltransferase ST6Gal-I regulates stem cell transcription factors and confers a cancer stem cell phenotype. Cancer Res. 76, 3978–3988
17. Lise, M., Belluco, C., Perera, S. P., Patel, R., Thomas, P., and Ganguly, A. (2000) Clinical correlations of α2,6-sialyltransferase expression in colorectal cancer patients. Hybridoma 19, 281–286
18. Wang, Y. C., Stein, J. W., Lynch, C. L., Tran, H. T., Lee, C. Y., Coleman, R., Hatch, A., Antontsev, V. G., Chy, H. S., O’Brien, C. M., Murthy, S. K., Laslett, A. L., Peterson, S. E., and Loring, J. F. (2015) Glycosyltransferase ST6Gal1 contributes to the regulation of pluripotency in human pluripotent stem cells. Sci. Rep. 5, 13317
19. Tateno, H., Toyota, M., Saito, S., Omura, Y., Ito, Y., Hiemori, K., Fukumura, M., Matsushima, A., Nakanishi, M., Ohnuma, K., Akutsu, H., Umezawa, A., Horimoto, K., Hirabayashi, J., and Asahima, M. (2011) Glycome diagnosis of human induced pluripotent stem cells using lectin microarray. J. Biol. Chem. 286, 20345–20353
20. Nairn, A. V., Aoki, K., dela Rosa, M., Porterfield, M., Lim, J. M., Kulik, M., Pierce, J. M., Wells, L., Dalton, S., Tiemeyer, M., and Moremen, K. W. (2012) Regulation of glycan structures in murine embryonic stem cells: combined transcript profiling of glycan-related genes and glycans structural analysis. J. Biol. Chem. 287, 37835–37856
21. Zhuo, Y., Chammass, R., and Bellis, S. L. (2008) Sialylation of β1 integrins
blocks cell adhesion to galectin-3 and protects cells against galectin-3-induced
apoptosis. J. Biol. Chem. 283, 22177–22185.
22. Fukumori, T., Takenaka, Y., Yoshii, T., Kim, H. R., Hogan, V., Inohara, H.,
Kagawa, S., and Raz, A. (2003) CD29 and CD27 mediate galectin-3-induced
type II T-cell apoptosis. Cancer Res. 63, 8302–8311.
23. Amano, M., Galvan, M., He, J., and Baum, L. G. (2003) The ST6Gal I
sialyltransferase selectively modifies N-glycans on CD45 to negatively regu-
late galectin-1-induced CD45 clustering, phosphatase modulation, and
T cell death. J. Biol. Chem. 278, 7469–7475.
24. Kitazume, S., Imamaki, R., Ogawa, K., Komi, Y., Futakawa, S., Kojima, S.,
Hashimoto, Y., Marth, J. D., Paulson, J. C., and Taniguchi, N. (2010) α2,6-
sialic acid on platelet endothelial cell adhesion molecule (PECAM) regu-
lates its homophilic interactions and downstream antiapoptotic signaling.
J. Biol. Chem. 285, 6515–6521.
25. Schultz, M. J., Swindall, A. F., Wright, J. W., Sztul, E. S., Landen, C. N., and
Bellis, S. L. (2013) ST6Gal-I sialyltransferase confers cisplatin resistance in
ovarian tumor cells. J. Ovarian Res. 6, 25.
26. Berven, L. A., and Crouch, M. F. (2000) Cellular function of p70S6K: a role
in regulating cell motility. Immunol. Cell Biol. 78, 447–451.
27. Pearson, R. B., and Thomas, G. (1995) Regulation of p70S6K/p85S6K and its
role in the cell cycle. Prog. Cell Cycle Res. 1, 21–32.
28. Piva, R., Belardo, G., and Santoro, M. G. (2006) NF-κB: a stress-regulated
switch for cell survival. Antioxid. Redox. Signal 8, 478–486.
29. Foster, D. A., Yellen, P., Xu, L., and Saqcena, M. (2010) Regulation of G1
cell cycle progression: distinguishing the restriction point from a nutrient-
sensing cell growth checkpoint(s). Genes Cancer 1, 1124–1131.
30. Hanahan, D., and Weinberg, R. A. (2011) Hallmarks of cancer: the next
generation. Cell 144, 646–674.
31. Normanno, N., De Luca, A., Bianco, C., Strizzi, L., Mancino, M., Maiello,
M. R., Carotenuto, A., De Feo, G., Caponigro, F., and Salomon, D. S. (2006)
Epidermal growth factor receptor (EGFR) signaling in cancer. Gene 366,
2–16.
32. Regad, T. (2015) Targeting RTK signaling pathways in cancer. Cancers 7,
1758–1774.
33. Eves, E. M., Xiong, W., Bellacosa, A., Kennedy, S. G., Tsichlis, P. N., Ros-
ner, M. R., and Hay, N. (1998) Akt, a target of phosphatidylinositol 3-ki-
nase, inhibits apoptosis in a differentiating neuronal cell line. Mol. Cell
Biol. 18, 2143–2152.
34. Clark, A. S., West, K., Streicher, S., and Dennis, P. A. (2002) Constitutive
and inducible Akt activity promotes resistance to chemotherapy, trastuz-
umab, or tamoxifen in breast cancer cells. Mol. Cancer Ther. 1, 707–717.
35. Kennedy, S. G., Wagner, A. J., Conzen, S. D., Jordan, J., Bellacosa, A.,
Tsichlis, P. N., and Hay, N. (1997) The PI 3-kinase/Akt signaling pathway
delivers an anti-apoptotic signal. Genes Dev. 11, 701–713.
36. Lu, J., Isaji, T., Im, S., Fukuda, T., Hashii, N., Takakura, D., Kawasaki, N.,
and Gu, J. (2014) β-Galactoside α2,6-sialyltransferase 1 promotes trans-
forming growth factor-β-mediated epithelial-mesenchymal transition. J.
Biol. Chem. 289, 34627–34641.
37. Karin, M., and Lin, A. (2002) NF-κB at the crossroads of life and death.
Nat. Immunol. 3, 221–227.
38. Hussain, A. R., Ahmed, S. O., Ahmed, M., Khan, O. S., Al Abdalmohsen, S.,
Platanias, L. C., Al-Kuraya, K. S., and Uddin, S. (2012) Cross-talk between
NFκB and the PI3-kinase/AKT pathway can be targeted in primary effu-
sion lymphoma (PEL) cell lines for efficient apoptosis. PLoS ONE 7,
e39945.
39. Dan, H. C., Cooper, M. J., Cogswell, P. C., Duncan, J. A., Ting, J. P., and
Baldwin, A. S. (2008) Akt-dependent regulation of NFκB is controlled by mTOR
and Raptor in association with 14-3-3. Genes Dev. 22, 1490–1500.
40. Baud, V., and Karin, M. (2009) Is NFκB a good target for cancer therapy?
Hopes and pitfalls. Nat. Rev. Drug Discov. 8, 33–40.
41. Seales, E. C., Jurado, G. A., Singhal, A., and Bellis, S. L. (2003) Ras oncogene
directs expression of a differentially sialylated, functionally altered β1 in-
tegrin. Oncogene 22, 7137–7145.
42. Dalziel, M., Dall’Olio, F., Mungul, A., Piller, V., and Piller, F. (2004) Ras
oncogene induces β-galactoside α2,6-sialyltransferase (ST6Gal I) via a
RalGDF-mediated signal to its housekeeping promoter. Eur. J. Biochem.
271, 3623–3634.
43. Boumahdi, S., Driessens, G., Lapouge, G., Rorive, S., Nassar, D., Le Mer-
cier, M., Delatte, B., Cauwe, A., Lenglez, S., Nkusi, E., Brohéé, S., Salmon,
I., Dubois, C., del Marmol, V., Fuxs, F., et al. (2014) SOX2 controls tumour
initiation and cancer stem-cell functions in squamous-cell carcinoma.
Nature 511, 246–250.
44. Lee, M., Park, J. J., and Lee, Y. S. (2010) Adhesion of ST6Gal I-mediated
human colon cancer to fibronectin contributes to cell survival by integrin β1-
mediated paxillin and AKT activation. Oncol. Rep. 23, 757–761.
45. Chen, X., Wang, L., Zhao, Y., Yuan, S., Wu, Q., Zhu, X., Niang, B., Songs,
S., and Zhang, J. (2016) ST6Gal-I modulates docetaxel sensitivity in hu-
nan hepatocarcinoma cells via the p38 MAPK/caspase pathway. Nature
Comm. 7, 22177–22187.
46. Beck, B., and Blanpain, C. (2013) Unravelling cancer stem cell potential.
Nat. Rev. Cancer 13, 727–738.
47. Tavalc, R. T., Hart, L. S., Dicker, D. T., and El-Deiry, W. S. (2007) Effects
of low confluency, serum starvation and hypoxia on the side population of
cancer cell lines. Cell Cycle 6, 2554–2562.
48. Lin, S. P., Lee, Y. T., Wang, J. Y., Miller, S. A., Kato, H., Hung, M. C., and
Hung, S. C. (2012) Survival of cancer stem cells under hypoxia and serum
depletion via decrease in PP2A activity and activation of p38-MAPK/caspase
pathway. Onco-target 7, 51955–51964.
49. Liu, Y. C., Yen, H. Y., Chen, C. Y., Chen, C. H., Cheng, P. F., Yuan, Y. H.,
Chen, C. H., Khoo, K. H., Yu, C. J., Yang, P. C., Hsu, T. L., and Wong, C. H.
(2011) Sialylation and fucosylation of epidermal growth factor-receptor
suppress its dimerization and activation in lung cancer cells. Proc. Natl.
Acad. Sci. 108, 11332–11337.