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ST6GAL1: A key player in cancer (Review)

REBECCA GARNHAM, EMMA SCOTT, KAREN E. LIVERMORE and JENNIFER MUNKLEY

Institute of Genetic Medicine, Newcastle University, International Centre for Life, Newcastle Upon Tyne NE1 3BZ, UK

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Abstract. Aberrant glycosylation is a universal feature of cancer cells and there is now overwhelming evidence that glycans can modulate pathways intrinsic to tumour cell biology. Glycans are important in all of the cancer hallmarks and there is a renewed interest in the glycomic profiling of tumours to improve early diagnosis, determine patient prognosis and identify targets for therapeutic intervention. One of the most widely occurring cancer associated changes in glycosylation is abnormal sialylation which is often accompanied by changes in sialyltransferase activity. Several sialyltransferases are implicated in cancer, but in recent years ST6 β -galactoside α -2,6-sialyltransferase 1 (ST6GAL1) has become increasingly dominant in the literature. ST6GAL1 catalyses the addition of α 2,6-linked sialic acids to terminal N-glycans and can modify glycoproteins and/or glycolipids. ST6GAL1 is upregulated in numerous types of cancer (including pancreatic, prostate, breast and ovarian cancer) and can promote growth, survival and metastasis. The present review discusses ST6GAL1 in relation to the hallmarks of cancer, and highlights its key role in multiple mechanisms intrinsic to tumour cell biology.

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1. Introduction

Glycosylation is an enzymatic process that links glycan sugars to other glycans, lipids or proteins and is essential to virtually every biological process (1). The complete pattern of glycan

modifications in a cell or tissue (known as ‘the glycome’) is assembled by the synchronised action of numerous glycosylation enzymes on glycoproteins and/or lipids (2). Changes to the glycome are well documented in cancer, and aberrant glycosylation is not just a consequence, but also a driver of a malignant phenotype (3). The hallmarks of cancer were originally described in 2000 and refer to capabilities acquired during the multi-step development of cancer to enable cancer cells to survive, proliferate and metastasise (4). They include sustaining proliferative signalling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Underpinning these hallmarks are genome instability and inflammation which contribute to multiple hallmark capabilities (4). In 2011 two next generation cancer hallmarks were proposed (reprogramming of energy metabolism and evading immune destruction) and the ‘tumour microenvironment’ was recognised as contributing to the acquisition of hallmark traits (5).

Although not included in the original and next generation hallmarks, aberrant glycosylation is now also widely recognised as a new hallmark of cancer causally associated with all of the hallmark capabilities (2,3,6). One of the most widely occurring cancer associated changes in glycosylation is abnormal sialylation, which is often driven by the altered expression of sialyltransferase enzymes (7-9) and is linked to poor patient prognosis and metastasis (10-13). Several sialyltransferase enzymes are implicated in cancer, but in recent years ST6GAL1 (which catalyses the addition of α 2,6-linked sialic acids onto terminal N-glycans) has become increasingly dominant in the literature. ST6GAL1 is upregulated in numerous types of cancer, including pancreatic, prostate, breast and ovarian cancer, and it has key roles in tumour aggression and metastasis (14-18). Here, we discuss ST6GAL1 in the context of the original and emerging hallmarks of cancer, and highlight its role in pathways intrinsic to tumour cell biology (Fig. 1).

2. Altered ST6GAL1 in cancer

ST6GAL1 levels are upregulated in several carcinomas, as is the degree of α 2,6-sialylation (15-17,19-26) (Table I). In particular, elevated ST6GAL1 is often correlated with high tumour grade, metastasis and reduced patient prognosis. In both prostate and breast cancer, ST6GAL1 expression correlates with a more aggressive tumour grade (16,27), and in ovarian cancer levels increase in advanced stage disease (18). Tumours with elevated

Correspondence to: Dr Jennifer Munkley, Institute of Genetic Medicine, Newcastle University, International Centre for Life, Central Parkway, Newcastle Upon Tyne NE1 3BZ, UK
E-mail: jennifer.munkley@ncl.ac.uk

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ST6GAL1 expression are thought to be more invasive and metastatic, evidenced by increased lymphovascular invasion, deep stromal invasion, distant metastasis and neighbouring vesicle invasion (16,28,29). ST6GAL1 is also associated with reduced recurrence-free intervals and a poorer overall survival in ovarian, prostate and pancreatic cancer (16,18,28,30). The only exception is bladder cancer, where ST6GAL1 is believed to have a tumour suppressive role (31).

3. ST6GAL1 in the hallmarks of cancer

Activating invasion and metastasis. The ability of cancer cells to invade and spread is central to the development of an invasive, malignant tumour. This development, which promotes local invasion and distant metastasis is a multistep process referred to as the invasion-metastasis cascade (5). The process is regulated via complex crosstalk across several signalling pathways and transcription factors, resulting in an epithelial to mesenchymal transition (EMT) of cancer cells (32). EMT is an example of cellular phenotype switching, characterised by a loss of epithelial markers in favour of the migratory phenotype of mesenchymal cells (33,34). The literature demonstrating a relationship between sialylation and the acquisition of invasive and metastatic phenotypes is extensive (13,35-41). Further to this, ST6GAL1 upregulation has been shown to induce a more invasive, migratory cell phenotype in gastric, colon, liver, prostate, ovarian, breast and cervical cancers (16,17,42,43). ST6GAL1 gene knockdown and over expression experiments *in vitro* have demonstrated its capacity to regulate the invasive and metastatic features of cancer cells in several cell types (16,24,35,42-44). In 2017, a prominent glyco-oncology study identified elevated ST6GAL1 as part of a pro-metastatic gene signature in melanoma tumours (29). ST6GAL1 is also increased in patients with metastatic cervical cancer (7,43), where levels correlate with stromal invasion, metastatic spread to the lymph nodes and poor patient prognosis (43). Similarly, in triple-negative breast cancer, ST6GAL1 levels are linked to metastasis and reduced survival times (19), and high ST6Gal1 in ovarian cancer is associated with lymphovascular invasion and distant metastasis (7). In breast cancer cells, overexpression of ST6GAL1 increases the turnover of cell surface E-cadherin and promotes TGF- β -induced EMT providing a potential mechanistic link between ST6GAL1-mediated sialylation and metastasis (17).

Sustained proliferative signalling. Central to cancer cell biology is the excessive capacity to proliferate, and to do so in the absence of proliferative stimuli (4,45). Sialylation has been shown to alter proliferative signalling cascades in different cancers (24,46). ST6GAL1 in particular can regulate cellular proliferation in hepatocellular carcinoma (HCC), as shown through gene knockdown and overexpression studies carried out in the MHCC97L HCC cell line. The HCC *in vitro* studies suggested that overexpression of ST6GAL1 in HCC cells increased activation of the PI3K/Akt signalling pathway (42). Hyper-activation of the PI3K signalling cascade is well documented in several different cancer subtypes as regulating and promoting hyper-proliferation of oncogenic cells (47-50). If ST6GAL1 can activate PI3K/Akt signalling, as suggested by Zhao *et al* (42) this may point towards mechanistic link

between ST6GAL1 upregulation and increased cellular proliferation. Similar effects on proliferation and PI3K/Akt signalling have also been observed following ST6GAL1 gene silencing in the DU145 and PC-3 prostate cancer cell lines. Wei *et al* (16) observed approximately a two-fold decrease in cancer cell proliferation following ST6GAL1 gene silencing. It is important to note that this relationship has only been observed in HCC and prostate cancer, and is contradicted by findings generated using glioma cells, assessing the relationship between ST6GAL1 and proliferative capacity. In this instance, ST6GAL1 overexpression did not perpetuate hyper-proliferation (23). This contradiction could suggest that the effect of ST6GAL1 on proliferative signalling may be exclusive to specific cancer subtypes.

Enabling replicative immortality. In the seminal 2000 'hallmarks of cancer' paper, replicative immortality was outlined as being essential to develop and sustain macroscopic tumours (4,5). This innate replicative potential, without the threat of cellular senescence has been termed immortalization and is underpinned by telomere abnormalities or oncogenic induced cellular senescence. Several key oncogene or tumour suppressor genes have a role in promoting oncogenic induced cellular senescence (including *Tp53*, *RAS*, *c-MYC* and *PTEN*) and some have been shown to be targets for glycosylation (51-56). As outlined previously, ST6GAL1 can regulate PI3K/AKT signalling, a known RAS effector cascade (16,57). ST6GAL1 gene knockdown in prostate cancer cells results in decreased levels of *PI3K/AKT/GSK-3 β* and β -catenin signalling molecules (16). B-catenin, a member of the Wnt signalling pathway, has a well-defined oncogenic activity and has been well characterised as an enabler of replicative immortality through direct activation of telomerase reverse transcriptase (TERT) (58,59). Sialylation by ST6GAL1 upregulates several oncogenes crucial for the immortalization of cancer cells, interacting with RAS effector pathways, and Wnt/ β -catenin signalling. Known downstream effects of these pathways result in telomerase repression and oncogenic induced stress, thereby indicating a role for ST6GAL1 in enabling replicative immortality (52).

Sustained angiogenesis. For a cancer to sustain macroscopic tumour development and promote metastasis, the formation of neovasculature is necessary for the supply of nutrients and oxygen. This process, known as angiogenesis, is tightly regulated by opposing factors; stimulating and inhibiting the receptors displayed on the surface of vascular endothelial cells (4,5,60,61). Although key molecular regulators of angiogenesis have been identified, such as VEGF and TSP-1, it is now widely accepted that regulation of angiogenesis is a highly complex process, heavily influenced by the tumour microenvironment, gaining influence from things such as tumour metabolism, immune infiltrate and cancer-associated fibroblasts (CAFs) (62). Abnormal glycosylation changes have been identified throughout several pro-angiogenic pathways in cancers, and recently it was found that VEGF-induced angiogenesis was dependent upon sialylation of the VEGF-receptor 2 (VEGFR2) (63-68). A high profile Cell paper, published in 2014, concluded that cancer cells can undergo hypoxia induced glycan remodelling which can

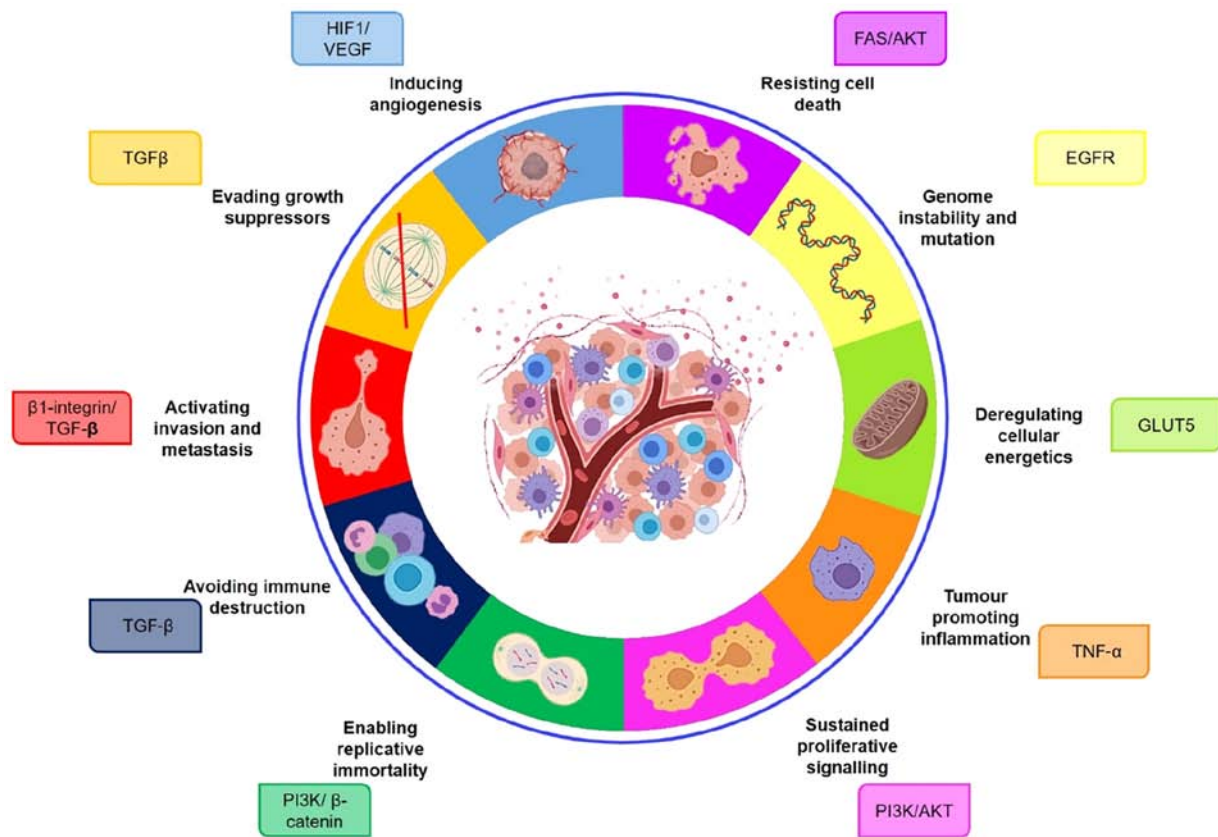


Figure 1. The role of ST6 β -galactoside α -2,6-sialyltransferase 1 in the hallmarks of cancer. Image created using BioRender.

confer tumour resistance to anti-VEGF treatment. Levels of ST6GAL1 were elevated in tumours sensitive to anti-VEGF treatment, and *ST6GAL1* knockdown protected tumours from anti-VEGF treatment (22,67). In line with this, knockdown of *ST6GAL1* in an osteosarcoma cell line also reduced levels of VEGF (22), suggesting a major role for ST6GAL1 in cancer associated angiogenesis.

Tumour metabolism and hypoxia play a key part in promoting the formation of new blood vessels through the activation of several pro-angiogenic factors (62,69). The major determinant of hypoxia mediated angiogenesis is HIF1, a protein capable of upregulating VEGF and PI3K signalling in the absence of oxygen to promote the growth of new vessels (69,70). Hypoxia experiments carried out in ovarian and pancreatic cancer cell lines indicate increased *ST6GAL1* expression can lead to an accumulation of HIF1 α under hypoxic conditions, as well as increases in HIF1 α transcriptional targets (20). These increases suggest upregulated ST6GAL1 confers pro-survival characteristics under hypoxic conditions. Taken alongside evidence of ST6GAL1 as a major regulator of VEGF signalling, this suggests ST6GAL1 is an important sialyltransferase critical for angiogenesis.

Resisting cell death. It is well established that for a cancer to develop it must evade and overcome cellular apoptosis (71-73). Apoptosis is the programmed death of a cell, central for ensuring the correct cell turnover and development, so much so that aberrant apoptosis has been implicated in several human diseases, including cancer (74). For this reason, research into the mechanisms which underpin cell death has

exploded over the past two decades, and we now have a fair understanding of the prominent molecular processes which regulate apoptosis (75). Glycan changes have been linked to apoptosis since the late 1990s (76-80), and sialylation (including ST6GAL1 mediated sialylation) has been functionally associated with the programmed cell death of several different cell types (21,81-83). The TNF family of death receptors regulate programmed cell death, and include proteins such as DR4, DR5 and FAS. In a colon cancer model, ST6GAL1 upregulation can decrease levels of FAS mediated cell death (independent of both DR4 and DR5) through direct sialylation of the FAS protein (84). As mentioned earlier, elevated levels of ST6GAL1 drive hyper-activated AKT signalling in several cancer models, a key pathway which can be upregulated to enable tumour cells to evade apoptosis (85,86). Due to the large number of downstream targets of PI3K signalling, the effect of ST6GAL sialylation on this pathway may result in changes in both cell survival and cellular proliferation. This, taken with evidence that a key TNF death receptor is a direct target of ST6GAL1, indicates that ST6GAL1 upregulation can confer anti-apoptotic characteristics.

Evading growth suppressors. Tumour suppressor genes negatively regulate cell proliferation and tumour growth and are vital 'gate-keepers' of the genome (87). A key feature of cancer cells is their ability to inactivate or avoid these growth suppressing signals to continue hyper-proliferation (4,5). Several known tumour suppressor genes have been identified as targets of abnormal glycosylation, thereby promoting tumorigenesis (2,88). Although in all other cancers sialylation

Table I. Studies on ST6GAL1 in cancer.

Author, year	Cancer	Role of ST6GAL1	Characteristics	(Refs.)
Antony <i>et al</i> , 2014	Bladder	Downregulated	Invasive, high grade tumours with metastasis	(31)
Lu <i>et al</i> , 2014	Breast	Upregulated	Metastatic disease	(17)
Wang <i>et al</i> , 2003	Cervical	Upregulated	Stromal invasion with malignant disease	(43)
Swindall <i>et al</i> , 2011; Chiricolo <i>et al</i> , 2006; Schultz <i>et al</i> , 2016	Colon	Upregulated	Invasive, aggressive disease with chemoresistance	(84,107,108)
Gretschel <i>et al</i> , 2003	Gastric	Upregulated	Metastatic disease	(109)
Yamamoto <i>et al</i> , 2001	Glioma	Upregulated	More advanced invasive disease linked to metastasis	(23)
Ma <i>et al</i> , 2015	Leukemia	Upregulated	Increased chemoresistance	(110)
Zhao <i>et al</i> , 2014; Pousset <i>et al</i> , 1997	Liver	Upregulated	More aggressive, invasive disease with chemoresistance	(42,111)
Agrawal <i>et al</i> , 2017	Melanoma	Upregulated	Metastatic disease	(29)
Wang <i>et al</i> , 2005; Wichert <i>et al</i> , 2018	Ovarian	Upregulated	Advanced invasive	(7,18)
Hsieh <i>et al</i> , 2017	Pancreatic disease with distant metastasis	Upregulated	Advanced metastatic disease	(15)
Wei <i>et al</i> , 2016; Munkley <i>et al</i> , 2016	Prostate	Upregulated	Poor patient prognosis and metastatic disease	(16,26)

ST6GAL1, ST6 β -galactoside α -2,6-sialyltransferase 1.

by ST6GAL1 appears to be a pro-oncogenic event, upregulation of the sialyltransferase in bladder cancer appears to have a tumour suppressive role, with low ST6GAL1 expression being a feature of more advanced invasive disease, and upregulation of ST6GAL1 a hallmark of non-invasive disease (31). This obvious contradiction with evidence from other cancer types highlights the heterogeneous role of ST6GAL1 activity in cancer. It is useful to note the functional association between ST6GAL1 directed sialylation and TGF β signalling, as TGF β has been identified as both a tumour suppressor gene and a cytokine capable of promoting oncogenic events (89). The complex nature of cancer disease biology and of sialylation may mean that ST6GAL1 has dual roles in both promoting and inhibiting cancer progression.

The enabling characteristics and emerging hallmarks of cancer. In 2011, Hanahan and Weinberg revisited their original hallmarks of cancer and proposed two emerging hallmarks, 'deregulating cellular energetics' and 'avoiding immune destruction'. They also identified two enabling characteristics, 'promoting inflammation' and 'genome instability and mutation' as crucial in the acquisition of the cancer hallmarks (5). Consistent with the previous hallmarks of cancer, ST6GAL1 appears to have important interactions with pathways important in the 'next generation' hallmarks of cancer. An anomaly of cancer cell biology is that even in the presence of oxygen, cancer cells will fuel themselves using aerobic glycolysis—a phenomenon now termed the 'Warburg effect' (90). This metabolic reprogramming allows cancer cells to thrive and meet the

energetic demands of their proliferative capacity, and has been shown to be associated with changes in glycosylation (91,92). This need to proliferate often leaves cells in a glucose deficit, at which point other sugar substrates are utilised to sustain tumour growth (93). High dietary intake of fructose has been linked to increased risk of pancreatic cancer, and also linked to metastatic pancreatic cancer (15). In the same study, ST6GAL1 was found to be increased in metastatic disease, in a fructose dependant manner and through regulation by the GLUT5 fructose receptor. This link with GLUT5 suggests a possible link to sialylation by ST6GAL1 and metabolic reprogramming of cancer cells.

The ability of cancer cells to avoid immune destruction, through activation of immune suppressors allows for uninterrupted tumour growth and progression (94). As mentioned earlier, there is a mechanistic link between TGF- β signalling and ST6GAL1 directed sialylation. TGF- β is a known immunosuppressor gene, important in the regulation of helper T-cells and regulatory T-cells, inhibiting cytokine production and suppressing macrophages, dendritic cells and natural killer cells (95,96). Although the association between TGF- β and ST6GAL1 is better understood in the context of EMT, there may be a role for sialylation in allowing cancer cells to evade immune destruction through this TGF- β interaction. At odds with the idea that immune cells seek to destroy cancer cells, the evidence now suggests that some infiltrating immune cells promote tumourigenesis, contributing growth factors, survival factors and pro-angiogenic factors which all help to sustain the tumour microenvironment (97-99). Glycosylation, and in

particular sialylation is known to play an important role in regulation of the immune response (100,101). ST6GAL1-null mice exhibit a widespread immunodeficient phenotype, indicating that ST6GAL1 sialylation is integral to regulation of the immune system (102). ST6GAL1 has been shown to promote B cell activation. IgG has also been shown to be a direct target for ST6GAL1 sialylation in an estrogen dependant manner in rheumatoid arthritis (103). It is evident that immune signalling is a substrate for regulation by sialylation, however much more needs to be done to characterise the effect of ST6GAL1 on the immune system (104). Regulation of the immune response through aberrant sialylation could result in tumour promoting inflammation.

Genomic instability and an accumulation of genomic mutations underpin carcinogenesis in all cancer subsets. In an attempt to avoid instability, DNA damage sensors actively survey the genome for DNA damage, and upon recognition of mutations activate DNA repair pathways. Mutation or inactivation of these repair pathways, such as the p53 signalling pathway, will inevitably result in mutational accumulation. An upstream regulator of DNA repair pathways is EGFR, known to regulate DNA repair, DNA replication and maintenance of genome stability when found in the nucleus (105). A study of ST6GAL1 sialylation in ovarian cancer, suggested that increased ST6GAL1 sialylation confers resistance to chemotherapeutic intervention (106). Of interest, they postulated that this chemoresistance was through direct sialylation of EGFR by ST6GAL1, resulting in heightened activation of EGFR. This suggests a direct mechanistic link between ST6GAL1-sialylation and DNA damage repair and implicates ST6GAL1 in the maintenance of genome stability.

4. Conclusions and future perspectives

The hallmarks of cancer are crucial to our understanding of cancer cell biology, and in guiding our efforts to identify novel biomarkers and develop new therapeutic strategies. Aberrant glycosylation is a universal feature of cancer cells, and glycans can modulate several of the pathways intrinsic to tumour cell biology. Here, we suggest that the sialyltransferase enzyme ST6GAL1 has widespread applications in the study of cancer, and importantly is implicated in all of the recognised cancer hallmarks (Fig. 1). Given the widespread impact of sialylation in cancer, and the evident prognostic value of ST6GAL1 levels, an improved understanding of how ST6GAL1 mediated sialylation sustains cancer cell biology may open the door to a new range of cancer therapeutics.

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Authors' contributions

RG, ES, KEL and JM jointly conceived and designed the review, researched the literature and wrote the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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