Role of GD3 Synthase ST8Sia I in Cancers

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GD3 synthase controls the biosynthesis of complex gangliosides, bearing two or more sialic acid residues. Disialylated gangliosides GD3 and GD2 are tumor-associated carbohydrate antigens (TACA) in neuro–ectoderm-derived cancers, and are directly involved in cell malignant properties, i.e., migration, invasion, stemness, and epithelial–mesenchymal transition. Since GD3 and GD2 levels are directly linked to GD3 synthase expression and activity, targeting GD3 synthase appears to be a promising strategy through which to interfere with ganglioside-associated malignant properties.

ganglioside GD3 synthase epithelial–mesenchymal transition transcriptional regulation

1. Introduction

Changes in glycosylation is a common feature of cancer cells that affects both *N*- and *O*-glycosylproteins as well as glycophingolipids, leading to the expression of tumor-associated carbohydrate antigens (TACA). These changes in glycosylation are usually explained by changes in the expression of specific glycosyltransferases, and are associated with increased aggressiveness of the tumors and a poor prognosis for the patients [1]. Gangliosides constitute a subclass of glycophingolipids substituted by one or more sialic acid residues. In humans, sialic acid molecules are exclusively *N*-acetyl-neuraminic acids (Neu5Ac) that can be *O*-acetylated, mainly on C9 [2]. Gangliosides are essential compounds of the plasma membrane, notably expressed at the outer leaflet in microdomains named “glycosynapses”, where they interact with cholesterol, phospholipids, transmembrane receptors, and signal transducers, controlling carbohydrate-dependent cell adhesion and signaling [3]. Normal human tissues mainly express mono-sialyl gangliosides, such as GM3 or GM1a; alternatively, di- or tri-sialyl gangliosides, with two or three sialic acid residues linked to the Gal residue of lactosylceramide (LacCer-Gg_2Cer), are essentially found in developing tissues, during embryogenesis, and are mainly restricted to the nervous system in healthy adults [4]. Within glycosynapses, gangliosides are important regulators of receptor tyrosine kinases (RTK), and therefore play major roles in cell proliferation, adhesion, and motility [5]. Basically, mono-sialyl gangliosides are usually considered as downregulators of RTK signaling, whereas di-sialyl gangliosides upregulate RTK activation and downstream signaling pathways [6]. In mammals, the expression of b- and c-series gangliosides increases under pathological conditions, including cancers [7]. Particularly, di-sialyl gangliosides GD3 and GD2 have been described as TACA in neuroectoderm-derived tumors, including melanoma, neuroblastoma, and glioblastoma, as well as in breast cancer [8]. Moreover, substantial evidence has demonstrated the implication of complex gangliosides in oncogenesis by mediating cell proliferation, migration, stemness, tumor growth, and angiogenesis, making di-sialyl gangliosides interesting therapeutic targets for cancer immunotherapy [9]. The overexpression of complex gangliosides in cancers is usually correlated with the upregulation of *ST8SIA1* gene

expression, which encodes the key enzyme for complex ganglioside synthesis, GD3 synthase (CMP-*N*-acetylneuraminate, GM3 α2,8-sialyltransferase, or ST8Sia I, EC 2.4.99.8, GD3S). Despite the role of GD2 synthase (encoded by the *B4GALNT1* gene) as the enzyme directly responsible for GD2 synthesis, GD3S is the rate-limiting enzyme of GD2 biosynthesis in breast cancer stem cells that have undergone epithelial–mesenchymal transition (EMT). In breast cancer stem cells, GD2 is considered as the ganglioside responsible for cancer cell stemness and metastatic properties, and various immunotherapy strategies targeting GD2 are used, or in development, for numerous cancer types [10][11]. However, since GD2 synthesis depends on GD3S expression levels, targeting GD3S could be a valuable therapeutic approach, in combination with these conventional therapies.

2. Role of GD3S in Cancer Progression and Metastasis

GD3S expression has long been associated with cancer progression and metastasis, especially in cancers of neuroectoderm origin; moreover, GD3 and GD2 are well-known melanoma- and neuroblastoma-associated antigens, respectively, playing key roles in cancer progression [8].

In terms of melanoma, the GD3 ganglioside has, for several decades, been known as a specific melanoma-associated carbohydrate antigen. Both GD3 and GD3S are absent in healthy human melanocytes, but show high expression in primary melanoma tissues, as well as in most melanoma cell lines, such as SK-Mel-28 [12][13][14]. Furthermore, highly metastatic cells show an increase in GD3S expression compared to poorly metastatic cells [15]. Inhibition of GD3S expression by antisense knockdown leads to a significant decrease in the expression of GD3 in hamster AbC-1 melanoma cells, and results in a marked decreased in tumor growth without affecting melanogenesis [16]. In addition, the stable transfection of GD3S cDNA in SK-Mel-28-N1 mutant cells that only express a-series gangliosides, leads to the conversion of GM3 into GD3, and the production of GD3S positive melanoma cells, which proliferate and migrate more than control cells [17][18]. These phenotypic changes are related to a high level of phosphorylation and activation of three major adaptor proteins: paxillin, p130Cas, and focal adhesion kinase (FAK) [18][19]. It was also shown that gangliosides, and the enzymes involved in their metabolism, are strictly interconnected with melanoma aggressiveness, and could represent a useful prognostic and diagnostic tool [20]. Moreover, the upregulation of GD3S and cell surface GD3 gangliosides was associated with human melanoma brain metastasis [21].

The expression of GTs implicated in ganglioside biosynthesis is also altered in brain tumors; therefore, the analysis of GT mRNA levels may be used for both diagnosis and prognosis. For example, a high expression of GD3S in glioma biopsies, together with a decrease in the expression of GM2/GD2 synthase, correlated with an increase in overall survival of patients [22].

The inhibition of the GD3 expression in rat F11 hybrid neuroblastoma cells by stable transfection with an antisense vector against the GD3S gene, was associated with reduced cell migration in vitro and reduced metastatic potential, in a nude mouse model [23]. In parallel, the overexpression of GD3S increases tumorigenicity and the invasion of rat glioma cells, whereas anti-GD3 mAb specifically inhibits tumor growth [24]. The stable transfection of GD3S cDNA into the U-251MG glioma cell line leads to the activation of Erk1/2, Akt, p130Cas, paxillin, and focal

adhesion kinase signaling molecules, enhancing invasion activity, motility, and proliferation capacity in low or no serum concentrations without cell cycle arrest, which was achieved by avoiding the accumulation of p16 and p21 [25]. On the contrary, the lack of GD3S attenuated the malignant properties of gliomas in a genetically engineered mouse model [26].

Two clinical studies performed on public databases of tissues samples of invasive breast cancer have shown that GD3S displayed higher expression among ER-negative breast cancer tumors, and its overexpression was associated with poor pathohistological grading in ER-negative tumors [27][28]. A higher expression of *ST8SIA1* and *MET* was also observed in the basal-like subtype of human breast tumors [29]. The expression of GD3S is also known to be upregulated in osteolytic MDA-MET metastatic breast cancer cells [30].

GD3S expression in MDA-MB-231 cells induces the accumulation of b- and c-series gangliosides (GD3, GD2, and GT3) at the cell surface of MDA-MB-231 breast cancer cells, together with the acquisition of a proliferative phenotype under serum-free conditions [29][31]. GD3S expression increases the malignant properties of breast cancer cells by the specific and constitutive activation of the c-Met receptor by GD2, and the subsequent Erk/MAPK and PI3K/Akt signaling pathways [32].

3. Role of GD3S in EMT and Stemness Properties

Recent research into cancer cell heterogeneity has identified a small population of cancer cells, known as cancer stem cells (CSC), with high plasticity capability, self-renewal properties, and the ability to regenerate tumors when injected into immunodeficient mice over several generations. Furthermore, it has been shown that CSCs are the driving cell population in term of cancer progression and metastasis development [33]. Interestingly, this particular cell population is slow-cycling, and contains some quiescent cells that could account for chemotherapy and radiotherapy resistance [34]; these cells may also be associated with specific mechanisms, such as high ROS scavenger expression [35] or enhanced DNA damage responses [36]. Interestingly, the cell population can exit from dormancy and enter in an active cell cycle. Although typically symmetrical, CSC division gradually switches to differentiation or asymmetric divisions, allowing for the repopulation of the “damaged” tumor. Another effect hindering treatment is the ability of this population of CSCs to spread to distant organs through the lymphatic system or blood vessels. CSC able to reach into or rebuilt a new sustaining niche, could also reactivate an active cell cycle, growing to generate metastasis, which accounts for cancer relapse [34].

Importantly, both the establishment of a CSC-supportive tissue niche and the epithelial–mesenchymal transition (EMT) have been shown to be favored by many physiological phenomena. One of the conditions prone to this is an inflammatory environment. Indeed, transforming growth factor (TGF)-β1, tumor necrosis factor (TNF), or cytokines (interleukin (IL)-6) could all trigger EMT within differentiated tumoral hepatocytes, induced by a retro-differentiation program processing cells with a CSC phenotype [37][38]. Similarly, secreted IL-6 participates in EMT initiation in breast carcinoma. During the acquisition of a mesenchymal phenotype and invasive properties, cells re-express CD44, which has also been associated with CSC-like subpopulation enrichment [39][40]. In this context, GD3S expression has been associated with EMT marker expression, promoting cell migration, cell adherence/adhesion,

and colony formation, reflecting an increase in malignant properties [41]. In parallel, GD3S can induce the expression of CSC markers, such as aldehyde dehydrogenase (ALDH) activity, and enhance the functional CSC property into mammosphere formation capability [42].

It has been demonstrated that the CSC population, defined as $CD44^{hi}/CD24^{lo}$, from human breast cancer patient samples and cell lines specifically express GD2, and could be used as a specific cell surface marker [42]. Indeed, *ST8SIA1* knockdown, reducing GD2/3 expression, induces the differentiation of CSCs towards a non-CSC phenotype, which could be highlighted by a functional assay as decreased formation. Most importantly, *ST8SIA1* knockdown annihilated tumorigenicity in an immunodeficient mice model, preventing tumor formation [43][41]. Furthermore, increased expression of GD3, induced by the constitutive activation of the c-Met signaling pathway, led to the enhancement of stem cell properties and an increase in metastatic potential [29]. In addition, GD3 and the EGF receptor have be observed to be colocalized in breast cancer stem cells; in this condition, GD3 participates to EGFR signaling activation. Interestingly, knocking down GD3S in MDA-MB-468 mammary carcinoma cells induces increased sensitivity, in vitro and in vivo, of the EGFR inhibitor gefitinib [42].

Yeh et al., highlighted that the GD3 ganglioside is overexpressed in neurospheres enriched with gastric cancer stem cell (GCSC) markers [44]. In an immuno-deficient mice model, sorted CD133+/GD3+ cells exhibited CSC properties with higher self-renewal potential, higher stemness gene expression panel (e.g., Nestin and Sox2), and, most importantly, higher tumorigenicity. Regarding GD3 synthase (GD3S) expression, Yeh et al., also found that GD3 ganglioside levels increased in neurospheres and human glioblastoma samples, but not in samples of normal brain tissues. Similar to the breast cancer model, stem cell-associated properties in glioma are affected by the inhibition of GD3S expression. Therefore, the use of neutralizing antibody targeting GD3 in vivo has been shown to induce cytotoxicity against GD3+ cells, which has a significant consequence on GBM tumor growth inhibition. Meanwhile, Woo et al., were able to validate the specific overexpression GD2 in glioma cancer stem cells compared to neural stem cells (NSC) [45]. Comparing GBM CSCs, with adult human NSCs as cell surface markers, Woo et al., confirmed the specific overexpression of GD2 in glioblastoma CSCs [45]. Nevertheless, evaluating GD2 as a specific CSC marker for cancer stemness, Woo et al., did not observed any difference in stemness properties between cells with or without GD2, using patient-derived glioblastoma primo-cultures. Both GD2+ and GD2- had similar functional characteristics to CSCs, shown using sphere formation capacity assay. Therefore, GD2 might not be useful as a therapeutic strategy to specifically target CSCs in glioblastoma.

In parallel, GD3S and GD2 expression are both significantly increased after the induction of EMT in transformed human mammary epithelial cells. On the other hand, GD3S inhibition, within mesenchymal breast cancer cells, disturbed EMT maintenance and prevented metastasis [46]. GD3S pharmaceutical inhibition, using triptolide (a small molecule specifically targeting GD3S), or transcription inhibition, using shRNA, prevents EMT initiation and maintenance initiated through Snail, Twist, and TGF-β1 signaling pathways; this alters the mesenchymal properties of SUM159 and MDA-MB-231, two claudin-low breast cancer cell lines. Interestingly, FOXC2, a key transcription factor for mediating several EMT pathways, binds to the GD3S promoter, regulating its expression. Therefore, the expression of GD3S drives EMT in cells, which maintain it through a retro-positive feedback loop via FOXC2

activation of the GD3S promoter. Finally, GD3S expression has been shown to correlate with adverse prognosis in patients with triple negative human breast tumors, known to be enriched in CSCs and mesenchymal cells.

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