Ganglioside GD3 synthase (GD3S), a novel cancer drug target

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Abstract Gangliosides are a class of important glycosphingolipids containing sialic acid that are widely distributed on the outer surface of cells and are abundantly distributed in brain tissue. Disialoganglioside with three glycosyl groups (GD3) and disialoganglioside with two glycosyl groups (GD2) are markedly increased in pathological conditions such as cancers and neurodegenerative diseases. GD3 and GD2 were found to play important roles in cancers by mediating cell proliferation, migration, invasion, adhesion, angiogenesis and in preventing immunosuppression of tumors. GD3 synthase (GD3S) is the regulatory enzyme of GD3 and GD2 synthesis, and is important in tumorigenesis and the development of cancers. The study of GD3S as a drug target may be of great significance for the discovery of new drugs for cancer treatment. This review will describe the gangliosides and their roles in physiological and pathological conditions; the roles of GD3 and GD2 in cancers; the expression, functions and mechanisms of GD3S, and its potential as a drug target in cancers.

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1. Introduction

Cancer has become a major public health problem in China and the world \(^{1,2}\). The incidence and mortality rates of cancer in China are still increasing \(^{3}\). Cancer is a multistep process which includes many processes, such as initiation, development, migration and metastasis \(^{4}\). Most patients with breast, lung, colon and ovarian cancer have been found to have metastatic colonies. Approximately 90% of patients with cancer died from cancer metastasis \(^{5}\). Conventional therapeutics are not effective for treatment of these cancers. Therefore, it is important to find novel drug targets for cancer treatment.

Gangliosides are present in many cells, especially in the nervous system \(^{6}\). Recent studies have shown that gangliosides GD3 and GD2 are involved and further play important roles in many cancers \(^{6,7}\). GD3 synthase (GD3S) is the only enzyme that regulates the biosynthesis of GD3 and GD2 and therefore exerts a significant effect on cancers. In this review, we present the classification of gangliosides, the physiological and pathological significance of gangliosides in humans, the roles of GD3 and GD2 in cancers, and the potential of GD3S to serve as a novel drug target in cancer.

2. Gangliosides

2.1. Definition and discovery of gangliosides

*N*-Acetylenuraminic acid, also known as sialic acid, is a carbohydrate released from brain glycolipids \(^{8}\). In general, glycolipid containing sialic acid is called ganglioside, also known as glycosphingolipid with sialic acid. Gangliosides are a subfamily of glycosphingolipids that contain one or more sialic acid residues, consisting of a hydrophilic sialic acid-containing oligosaccharide chain and a hydrophobic neuraminide. In 1935, Kroeker first found that gangliosides accumulated in the brains of children with Tay-Sachs disease \(^{9}\). Gangliosides were later found to be widely distributed in the cell membranes of animal tissues, especially in the central nervous system and in melanomas \(^{5,11}\).

2.2. Classification and nomenclature of gangliosides

Gangliosides can be divided into monosialo gangliosides (GM), disialogangliosides (GD) trisialogangliosides (GT) and tetrasiologanglioside (GQ) according to the number of sialic acid residues per molecule. Gangliosides are usually named by the Svennerholm nomenclature \(^{12}\). The capital letter "G" represents the ganglioside and the next letter "M, D, T, Q, P, H or S" represents the number of 1–7 sialic acids, respectively; the following number represents different glycosyl groups in gangliosides (Fig. 1). More than 70 kinds of gangliosides have been isolated and identified.

2.3. The physiological and pathological significance of gangliosides

Gangliosides are a family of acidic membrane glycolipids which exert important effects on cell development, differentiation and oncogenic transformation \(^{13}\). Gangliosides are sialic acid-containing glycolipids which are found on plasma membranes of nearly all vertebrate cells. GD3 plays essential roles in the development of normal brain and its levels are decreased in adults. In contrast, GD3 is upregulated in neuroectodermal and epithelial cancers \(^{14}\).

2.3.1. Roles of gangliosides in the central nervous system

Under normal physiological conditions, gangliosides are highly expressed in the central nervous system and promote the development of the nervous system \(^{15}\). Gangliosides are important for development of brain at early stages. They can promote the growth of neurons and axons, participate in the regulation of synapse plasticity, and are involved in the differentiation of neural cells. They are able to protect the function of nerves and promote regeneration of nerve by affecting certain cytokines as well \(^{16}\). Meanwhile, Gangliosides are also of great importance under pathological conditions. In Parkinson's disease, inhibition of ganglioside expression can reduce apoptosis of cells and protect nerves \(^{17}\). More than that, Gangliosides can also be targets for new drugs that inhibit β-amyloid aggregation to relieve Alzheimer's disease \(^{18}\).

2.3.2. Role of Gangliosides in cancers

In recent years, numerous studies showed that gangliosides are of significance in the occurrence and development of cancers. It was reported that the abnormal increase of gangliosides in serum is closely related to cancers \(^{19,20}\). So detection of ganglioside in serum could be used to predict prognosis and recurrence of patients with cancers \(^{21,22}\). Gangliosides exert many functions by different mechanisms in cancers. Gangliosides like GD3 and GD2 are found to be closely related to immunity. Ganglioside antigens on the surface of cancer cells, or shedding from the cells, act as immunosuppressors, as typically observed for the suppression of cytotoxic T cells and dendritic cells \(^{23}\). Gangliosides promote tumor-associated angiogenesis \(^{24,25}\) and strongly regulate cell adhesion/motility and thus initiate tumor metastasis \(^{26}\). Ganglioside antigens are directly connected with transducer molecules in microdomains to initiate adhesion. Ganglioside antigens and their catabolites are modulators of signal transduction by interacting with tyrosine kinases associated with growth factor receptors or other protein kinases \(^{27}\). Given the potential importance of these sialylated gangliosides and their modulating biological behavior in vivo, further studies on gangliosides are warranted.
3. Roles of GD3 and GD2 in cancers

Cancer is caused by both internal and environmental factors. Tumorigenesis is a multistep process including initiation, promotion and progression. The pathology of tumorigenesis is characterized by the accumulation of a multitude of alterations including genetic, cytogenetic, and epigenetic changes. Invasion and metastasis are the important hallmarks of cancers, which are also the biggest bottleneck in the treatment of cancers. Cancer stem cells, which are closely associated with initiation, development, invasion and metastasis of cancers, have the ability of self-renewal and differentiation. GD3 and GD2 are two very important gangliosides which exert many functions in tumorigenesis, development and metastasis. GD3 is the discovered earliest cancer-associated ganglioside that promotes adhesion and invasion of cancers. GD2 is used as a biomarker in a variety of cancer stem cells. GD3 and GD2 are highly expressed in a various malignant cancers and have become potential targets for next-generation cancer therapy.

3.1. The roles of GD3 in cancers

GD3 is a minor ganglioside in most normal tissues but plays a crucial role in the development of the brain. The expression of GD3 is significantly reduced in adults. However, expression of GD3 is increased in pathological conditions, such as cancers and neurodegenerative disorders.

GD3 mediates many functions in cancers by different mechanisms. The expression of GD3 is up-regulated in neoplastic cells and it mediates the invasion and survival of cancers. Intriguingly, GD3 can also regulate additional biological functions, such as cell proliferation and differentiation by stimulating expression of vascular endothelial growth factor (VEGF). Neuroectodermic cancers can mostly be characterized by the presence of cancer-associated glycosphingolipid antigens, such as gangliosides. GD3 is overexpressed in lymphangiomyomatosis (LAM). GD3 was also increased in plasma of children with T-cell acute lymphoblastic leukemia. In particular, GD3 is highly expressed in melanomas and small cell lung cancer cells, and its abnormal expression promoted the growth and invasion of the cancer cells. GD3 was isolated from the polar lipid fraction of ovarian cancer-associated ascites and functions as an inhibitory factor that prevents innate immune activation of natural killer T (NKT) cells. Purified GD3 displayed a high affinity for both human and mouse CD1d, which is involved in the presentation of lipid antigens to T cells. GD3 bound to the antigenic-binding site of CD1d and did not require additional processing for its inhibitory effect on NKT cells. Importantly, in vivo administration of GD3 inhibited α-galactosylceramide (α-GalCer)-induced NKT cell activation in a dose-dependent manner. Significant increases of gangliosides (GM3 and GD3) profile in human cerebrospinal fluid (CSF) were correlated with blood–brain barrier dysfunction including CSF hemorrhages and compressive syndrome, and some malignant processes (metastatic brain melanoma) as well. GD3 was found to be involved in formation of autophagosomes by interacting with core-initiator proteins of autophagy, such as autophagy and beclin 1 regulator 1 (AMBRA1) and WD repeat domain phosphoinositide-interacting protein 1 (WIPPI). GD3 was found to maintain the self-renewal capability of neural stem cells (NSCs) by interaction with EGF, which sustains the expression of EGF and its downstream signaling. Physical association between GD3 and neogenin was confirmed by immunoblotting of immunoprecipitates with anti-neogenin antibody from GD3 lysates. The intracytoplasmic domain of neogenin (Ne-ICD) was found in GD3 cells at higher levels than in GD3 cells when cells were treated with a proteasome inhibitor. Exogenous GD3 also induced Ne-ICD expression in GD3 cells. Overexpression of Ne-ICD in GD3 cells resulted in increased growth and invasion of the cells, suggesting that Ne-ICD plays a role as a transcriptional factor to drive malignant properties of melanomas after cleavage with γ-secretase. Knockdown of GD3 synthase resulted in the alleviation of tumor phenotypes and reduced activation levels of Src family kinase Yes whereas overexpression of GD3 enhanced the malignant properties of human melanoma cells by activating Yes. Ganglioside GD3 enhances signals of adhesion and augments malignant properties of melanoma cells by recruiting integrins to glycolipid-enriched microdomains. Apoptotic T cell levels from renal cell carcinoma (RCC) patients were higher than those from normal donors. GD3 plays a critical role in immune dysfunction observed in RCC patient T cells. GD3 regulates the apoptosis of activated but not resting T cells. The study from Ohkawa et al. showed that integrin-mediated signaling is essential in the effects of GD3 on the malignant properties of melanomas. Co-localization of GD3 and integrin at the focal adhesion supported these results. Overexpression of GD3 enhanced the association of focal adhesion kinase (FAK) with p130Cas after treatment of fetal calf serum. FAK as well as p130Cas and paxillin are crucial molecules undergoing stronger tyrosine phosphorylation in GD3-expressing melanoma cells. GD3 promotes cell growth and invasion through p130Cas and paxillin in malignant melanoma cells. GD3 expression in target cells can mediate the cytotoxicity of NK cells via siglec-7-dependent and -independent mechanisms. Monoclonal anti-GD3 antibodies were found to selectively inhibit the proliferation of human malignant glioma cells in vitro. Expression of neural cell adhesion molecules (NCAM) was reduced while GD3 expression was increased during the migratory phase of brain tumor invasion. Both NCAM and GD3 promoted motile behavior of cells in neoplastic glia. There is a correlation between advanced malignant melanoma and expression of GD3 antigen.
on patients’ peripheral T-lymphocytes. GD3 was highly expressed in hypervascularised areas of high grade gliomas, which support the involvement of the ganglioside in brain cancer angiogenesis. GD3 was also found to protect human melanoma cells from ionizing radiation-induced clonogenic cell death. A study from Koochekpour et al. showed that both TGF-β and gangliosides may act as indirect angiogenic factors by stimulating secretion of VEGF. GD3 was found to be associated with immunity. GD3 was identified as a suppressor of the innate immune response in ovarian cancer, and VEGF can potentiate immunosuppression mediated by GD3. GD3 is drug target of cancer treatment and antibody to GD3, ecromeximab (KW2871), was tested in a phase II study in clinical trials (Clinical trial number: NCT00679289).

3.2. The roles of GD2 in cancers

3.2.1. Expression of GD2 in the tumor

There is low GD2 expression in most normal tissues whereas high GD2 expression in many malignant cancers. In particular, GD2 is highly expressed in melanomas and small cell lung cancer cells, and its expression promotes growth and invasion of cells. GD2 is also highly prevalent in a cohort of breast cancer (BC) patients clustering cancer stem cell phenotype. GD2 may serve as a marker for neuroblastoma patients. The level of GD2 in cancer cells can be used as a prognostic biomarker in osteosarcoma. GD2 expression is maintained upon recurrence in patients with neuroblastoma. GD2 expression in many malignant cancers. In particular, GD2 is highly expressed in melanomas and small cell lung cancer cells, and its expression promotes growth and invasion of cells. GD2 is also highly prevalent in a cohort of breast cancer (BC) patients clustering cancer stem cell phenotype. GD2 may serve as a marker for neuroblastoma patients. The level of GD2 in cancer cells can be used as a prognostic biomarker in osteosarcoma.

3.2.2. Biomarker of cancer stem cell

Interaction of GD2 with growth factor receptors maintains breast cancer stem cell phenotype. GD2 may serve as a marker for identification and purification of murine bone marrow mesenchymal stem cells (mBM-MSCs). Meanwhile, Martinez et al. confirmed that cells selected by GD2 are a subpopulation of MSCs with features of primitive precursor cells. Human bone marrow mesenchymal stromal cells express the neural ganglioside GD2, which is a novel surface marker for the identification of MSCs. It was reported that GD2 identified a small fraction of cells in human breast cancer cell lines and patient samples that were capable of forming mammospheres and initiating tumors with as few as 10 GD2+ cells. In addition, the majority of GD2+ cells are also CD44+CD24-. The previously established cancer stem cell (CSC)-associated cell surface phenotype. Further study revealed that GD3S was highly expressed in GD2+, as well as in CD44+CD24- cells, and that interference with GD3S expression, either by shRNA or using a pharmacological inhibitor, significantly reduced the CSC population and CSC-associated properties.

3.2.3. Mechanisms of GD2 in cancer

GD2 is involved in the attachment of human melanoma and neuroblastoma cells to extracellular matrix proteins. GD2 enhanced platelet adhesion to extracellular matrix collagen by upregulating integrin α2β1-mediated tyrosine phosphorylation of p125FAK, thereby providing insight into how this interaction may be involved in neuroblastoma metastasis. The study from Ito et al. showed that GD2 could specifically bind siglec7 to promote the development of renal cell carcinoma.

3.2.4. Drug target of cancer immunotherapy

GD2 was found to be associated with immunity and anti-GD2 antibody was used as immunotherapy for neuroblastoma. In 2015, dinutuximab (an anti-GD2 mAb) was approved by the US Food and Drug Administration (FDA) and is currently used in a combination immunotherapeutic regimen for the treatment of children with high-risk neuroblastoma. However, dinutuximab can cause many adverse effects including neuropathic pain, hypotension, and fever, which limit its use. GD2 was also used as a therapeutic target for antibody-mediated therapy in patients with osteosarcoma.

4. GD3S, a novel drug target in cancer

4.1. Structure of GD3S

The gene coding for human GD3S is located in the p12 region of chromosome 12. In 1994, Sasaki et al. and Nara et al. isolated and cloned a GD3S cDNA, respectively. Transcripts of the GD3S gene encode a 356 amino acid protein that is mainly located in the Golgi apparatus. The first 29 amino acids are located in the cytoplasm. Amino acids 30–48 are helical structure and cross the membrane of Golgi. The remainder of the protein is in the Golgi chamber, which is the catalytic part of GD3S.

GD3S is an α-N-acetylgalactosaminide α-2,8-sialyltransferase. GD3S transfers the sialic acid residues from donor CMP-N-acetylgalactosaminide to the α-sialic acid residue of the receptor GM3 and thus generates GD3. GD3S is one of six members of the ST8Sia subfamily of sialyltransferases (ST8Sia I–VI), often referred as ST8Sia I. Sequence analysis reveals that the ST8Sia enzyme family can be divided into two groups: one is oligopolysialyltransferase including ST8Sia II, III, and IV; the other is a single sialyltransferase whose members are ST8Sia I, V and VI. The crystal structure of the ST8Sia III catalytic domain was resolved by Volkers et al. in 2015, which revealed the binding mechanism of its substrate and catalytic reaction mechanism. According to the similarities among ST8Sia subfamilies, the catalytic domain of GD3S can be obtained by comparing the primary amino acid sequences of ST8Sia I and ST8Sia III. It is also possible to speculate that some asparagine residues of GD3S may be glycosylated, such as N71, N119, N214 and N245. However, the precise three-dimensional structure of GD3S, the location and type of post-translational modification, the substrate recognition mechanism and the catalytic reaction mechanism still need further study.

4.2. GD3S expression in cancers

GD3 and GD2 are overexpressed in a variety of malignant cancers. GD3S is the only enzyme which regulates biosynthesis of GD3 and GD2 (Fig. 3). GD3S is highly expressed in many cancers and exerts various functions in initiation and development of cancers.

4.2.1. Breast cancer

CD44+CD24− is a biomarker of breast cancer stem cells. It was reported that GD3S was highly expressed in GD2+ breast cancer
stem cells, which were also CD44⁺ CD24⁻ breast cancer cells. Decreasing the expression of GD3S can inhibit proliferation and self-renewal of cancer stem cells. Inhibition of GD3S also reduced growth of tumor in vivo. A study showed that overexpression of GD3S could induce the accumulation of GD2 and GD3 in the membrane of the breast cancer cell line MDA-MB-231 and promote proliferation of cells by activating c-Met and the downstream targets MEK/ERK and the PI3K/AKT pathway. GD3S promoted metastasis of breast cancer by regulating epithelial mesenchymal transition (EMT). A study also showed that overexpression of GD3S increased the invasion and metastasis of breast cancer, and that metastasis of cancer was blocked by inhibiting the expression of GD3S.

4.2.2. Melanoma
GD3S is a specific antigen in melanoma. GD3S is overexpressed in many melanoma cell lines, such as M14 and SK-MEL-2 cells, and promote the development of melanoma. Hamamura et al. found that GD3S increased proliferation and invasion of cells by activating p130Cas, paxillin and the AKT pathway in melanoma. Overexpression of GD3S improved the adhesion of cancer cells to many kinds of extracellular matrixes and further promoted development of melanoma. GD3S was overexpressed and promoted proliferation and metastasis in melanoma cell line SK-MEL-2 and that NF-κB regulated transcription of GD3S and growth of cancers was inhibited when biosynthesis of GD3S was blocked.

4.2.3. Glioma
Glioma is a very malignant cancer with high recurrence. GD3S was also found to be highly expressed in patients with glioma. Many studies showed that CD133 could be used as a biomarker of glioma stem cells. However, CD133 was expressed not only in glioma stem cells, but also in many other cancers like colon cancer, retinoblastoma and leukemias. Yeh et al. studied the role of gangliosides in glioma stem cells and found that GD3 and GD2 were highly expressed in glioma stem cells. In consideration of the regulation of GD3S in the expression of GD3 and GD2, they further determined that combination of GD3S and CD133 could be used as a biomarker of glioma stem cells. GD3S promoted formation of spheres and the growth of glioma stem cells. Cancer was inhibited when expression of GD3S was interrupted, which suggested that GD3S played an essential role in glioma stem cells.

4.2.4. Other tumors
Ko et al. found that GD3S was highly expressed in lung cancer, and that inhibition of GD3S by siRNA reduced expression of GD2 and inhibited proliferation, migration and invasion of cells. Proliferation of PC12 cells, a rat pheochromocytoma, was increased and phosphorylation of TrkA and ERK1/2 activation was detected when cells were transfected with a GD3S expression plasmid, suggesting that cell proliferation was mediated by Ras/MEK/ERK. Overexpression of GD3S sensitized hepatocarcinoma cells to hypoxia and reduced tumor growth by suppressing the cSrc/NF-κB survival pathway. GD3S was overexpressed in melanomas and up-regulated in activated T lymphocytes. Cell migration, tumor growth and experimental metastasis of rat F-11 cells were reduced when expression of GD3S was suppressed.

4.3. Regulation of GD3S expression and potential as a drug target
Bobowska et al. identified the corresponding core promoter of GD3S in Hs578T breast cancer cells and showed that estradiol reduced mRNA expression of ST8SIA1 in estrogen receptor alpha (ERα) positive MCF-7 cells. The activity of the core promoter sequence of ST8SIA1 is also repressed by estradiol. Although there were two putative estrogen response elements (ERE) in the core promoter of ST8SIA1, they were not found to be involved in the promoter activity. Although NF-κB was found to be involved in ST8SIA1 transcriptional activation. Further study showed that estradiol prevented NF-κB to bind to the ST8SIA1 core promoter in ERα-expressing breast cancer cells by inhibiting the nuclear localization of p56 and p50. Triptolide (TPL) was found to down-regulate expression of GD3S through NF-κB activation in human melanoma cells. On the contrary, valproic acid induces transcriptional activation of human GD3S in SK-N-BE(2)-C human neuroblastoma cells. Kang et al. showed that the core promoter from −1146 to −646 was indispensable for expression of human GD3S, and that the core promoter lacked apparent TATA and CAAT boxes but contained putative binding sites for transcription factors c-Ets-1 (Protein C-Ets-1), CREB, AP-1 and NF-κB in human melanoma SK-MEL-2 cells. In addition, another study also indicated that NF-κB played an essential role in the transcriptional activity of human GD3S gene in Fas-induced Jurkat T cells. Furthermore, the translational activation of NF-κB-binding protein to the nucleus by Fas activation was also crucial for the increased expression of the GD3S gene in Fas-activated Jurkat T cells.

4.4. Study on an inhibitor of GD3S
Many studies showed that GD3S exerted significant functions in development of cancers. GD3S was also found to be related to immunity which was associated with initiation and development of cancers. The expression of GD3S in melanoma was associated with up-regulation in activated T lymphocytes. A study of small interfering RNAs for GD3S showed that GD3S could be used as a therapeutic target of lung cancers. Inhibition of GD3S can block biosynthesis of GD3 and GD2, thus reduces proliferation, migration and invasion of cancer cells. Therefore, GD3S may be used as potential drug target for many
cancers. Up to now, there are few studies on GD3S inhibitors. The only reported GD3S inhibitor is triptolide. Triptolide, an epoxy diterpene lactone, is one of the main active components extracted from the roots, leaves, flowers and fruits of the *Tripterygium*. It is a natural product with a variety of biological activities. Studies have shown that triptolide has anti-oxidation, and anti-rheumatism effects. It has also been reported that triptolide has a good therapeutic effect in pancreatic cancer, prostate cancer and bladder cancer. Kwon et al. found that triptolide inhibited cell proliferation by downregulating the expression of GD3S in human melanoma SK-MEL-2 cells. Sarkar et al. also found that triptolide inhibited the development of tumors by inhibiting the function of GD3S in breast cancer.

5. Summary and perspective

In recent years, great progress has been made in the study of gangliosides. As the key enzyme in the biosynthesis of GD3 and GD2, GD3S importantly functions in the development of the nervous system, the maintenance and repair of neural stem cells, the repair of nerves, and the growth, proliferation, infiltration and metastasis of cancers. The important roles of GD3S in cancers suggest that inhibitors of GD3S have good development and application prospects. GD3S inhibitors will be a focus of research on cancer drugs in the future. GD3S is highly expressed in a variety of cancers, but the regulation, especially the epigenetic regulation of its expression is still not clear. Bioinformatics analysis shows that methylation level of the GD3S promoter is very low. In addition, the mechanism of GD3S in cancers is still to be fully elucidated. Both GD3 and GD2 are found to be closely related to immunity. Currently there are few studies on GD3S and immune regulation. Therefore, the role of GD3S in cancer immunity needs further study in future. These studies on GD3S will be of great significance to understand the regulation of GD3S expression in cancers, its mechanism of action and the discovery of inhibitors.

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