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REVISION/REVIEW

Gangliosides generalities and role in cancer therapies

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ABSTRACT

Gangliosides are located in the plasma membrane; this confers them the ability to interact with other molecules in order to participate in important cellular processes. Some gangliosides presence or absence in the cell surface is associated with either normal condition or pathologies. Particularly in cancer, gangliosides play a critical role in pathological events like cellular malignancy, tumor formation, and metastasis, defining gangliosides as good candidates to be used as cellular markers. When specific gangliosides are exhibited, immunotherapy could be applied in order to inhibit tumorigenesis or induce an immunogenic response. Novel cancer treatments such as NGcGM3/VSSP vaccines, valproic acid, BMS-345541 inhibitor of GD2 and immunotherapies using 1E10 and 14F7 monoclonal antibodies are described. On this review, there will be studied the gangliosides that allowed developing biological techniques that can give immunogenicity to cancer cells.

Keywords: Gangliosides, cellular markers, cancer, vaccines, immunotherapy.

INTRODUCTION

The study of gangliosides as membrane molecules is not a new scientific interest. In fact, they were discovered as membrane glycolipids in the 1930s by Professor Klenk, who also noted that the content of these glycolipids was higher in the grey matter than in the white matter of the brain. Klenk proposed these molecules as gangliosides, because they were mainly present in the central nervous system cells, ganglienzellen[1,2]. Today, however, gangliosides are seen as attractive molecules to target cancer cells through immunotherapy[2]. This is because of its association with different oncogenic cellular processes[2–4]. Despite being highly expressed in the plasma membrane of eukaryotic cells (a-series mostly) at normal conditions[5,6], it has been reported that the expression of more complex gangliosides (b-series and c-series) increase in cancer cells, especially in those types of cancer from neuroectoderm origin, such as neuroblastoma, glioblastoma, melanoma, breast cancer, and lung cancer[7–9].

In general, gangliosides have been used in immunotherapy as the principal component in different vaccines, whose objective is to increase the immune response of patients towards specific gangliosides that are overexpressed in the membranes of cancer cells[10–12]. Some of these studies have shown good results[11–15]. In consequence, we consider them as promising proposals in cancer treatment. For this reason, the paper presented here has the purpose to make a ganglioside review on their chemical structure, physiology, biosynthesis and specially focusing on gangliosides and cancer relationship and their uses as a target for cancer immunotherapy.

Definition, structure and gangliosides location

Gangliosides, unlike other glycosphingolipids, contain one or more additional sialic acid molecules, thus they are considered as a subclass of acidic glycolipids. They usually present two variants of sialic acid, the N-Acetylated (NeuAc), which is normally found in healthy tissues of the body, and the N-Glycolylated (NeuGc) which

is the one that is overexpressed in the cell membranes of cancerous tissues[16,17]. Focusing on N-Glycolylated gangliosides, they are formed by one or more molecules of sialic acid, cholesterol, N-acetyl glucosamine molecules and negatively charged heads that are formed by oligosaccharides (Figure 1)[18].

The location of gangliosides are in the plasma membrane by crossing it or parallel to it, and the anchoring occurs in the ceramide group that is linked to the glycol hydrophilic head, which has sialic acid residues. This binding left free an exposed glycan on the cell surface. Gangliosides opportune location confers them the ability to interact with several extracellular molecules such as lipids, proteins, among others and they can also act as important receptors which are able to be recognized by the variant site of specific antibodies[2].

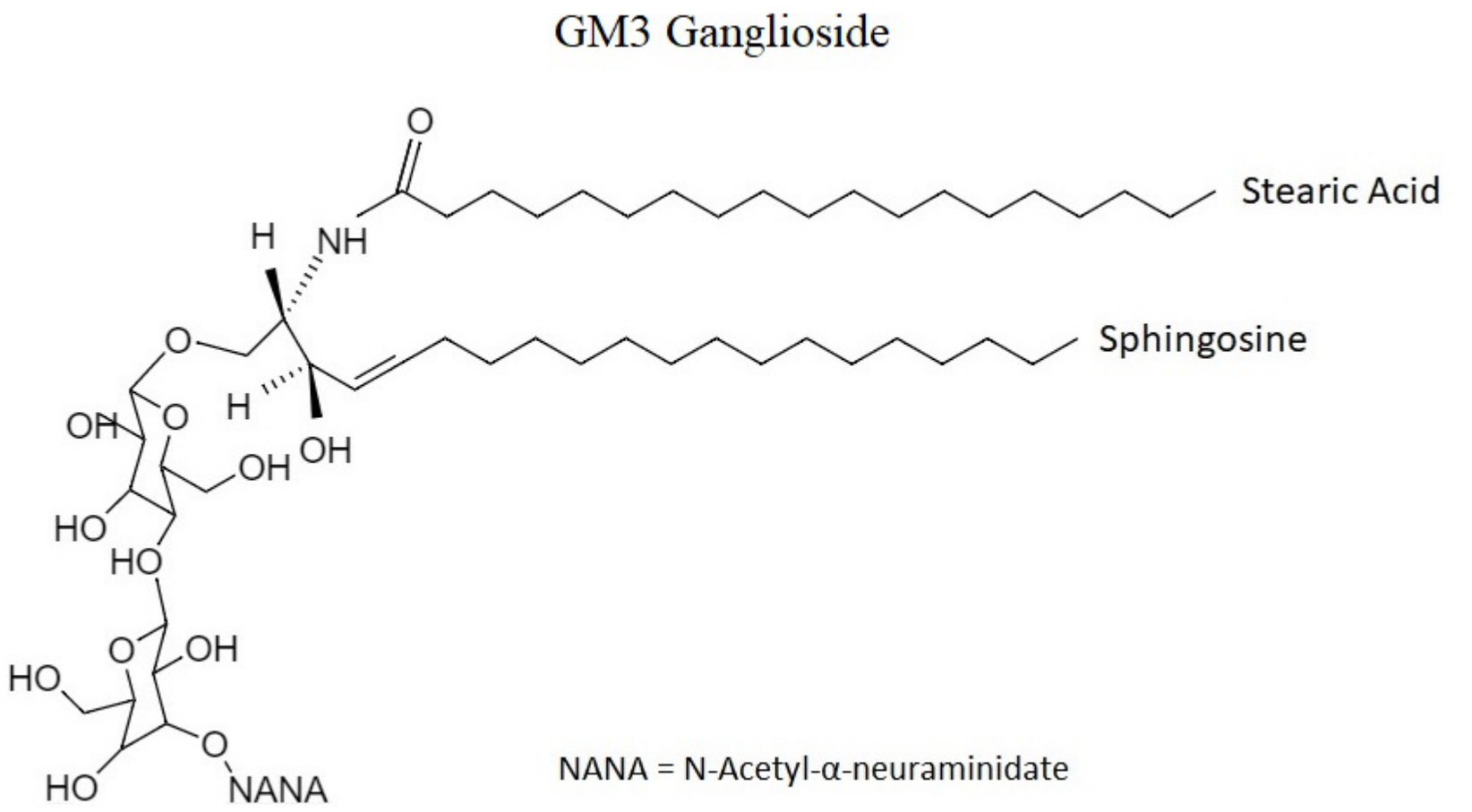

Figure 1: GM3 ganglioside structure.

Ganglioside biosynthesis pathways

Gangliosides are divided according to the number of sialic acid molecules that contain in their structure: 0-, a-, b- and c-series, which have 0, 1, 2 and 3 molecules of

sialic acid respectively . The synthesis of gangliosides starts in the endoplasmic reticulum, by the formation of ceramide (Cer synthase) , which then is transported to Cis-Golgi. The next step is led by the glycosyltransferases, whose function is the addition of monosaccharides, either glucose (GlcCer) or galactose (GalCer), to the ceramide in the 1- hydroxy group. The formation of LacCer is then produced by LacCer synthase, and α2,3-sialyltransferase ST3 Gal V (GM3) will transfer the first sialic acid residue to the previous molecule. Then, depending on the final ganglioside structure, sialyltransferases ST8Sia I (GD3S) and ST8Sia V (GT3 synthase) will be responsible for adding additional sialic acid molecules. Finally, different glycosylation steps of gangliosides will be done[7,9] (figure 2).

Some studies have suggested an association between the overexpression of complex gangliosides and the increase in the expression of the gene encoding GD3S[9,20].

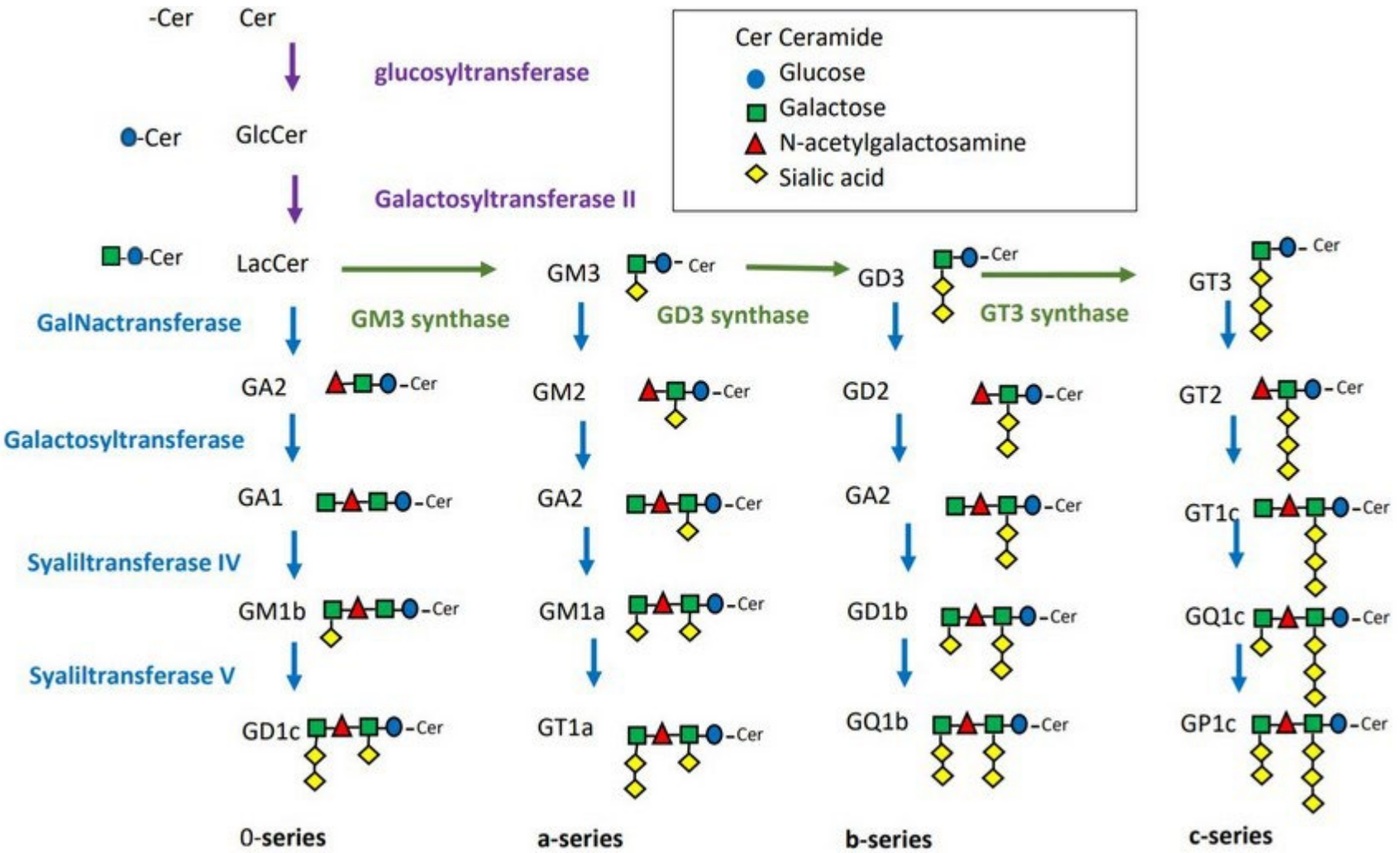

Figure 2: Ganglioside biosynthesis in eukaryotic cells, showing the intervention of different enzymes. The enzymes which transform Ceramide (Cer) to lactosylceramide (LacCer) (purple arrow), the enzymes (Glycotransferases add sugar and sialic acids) which form 0-series to c-series gangliosides (green arrow) and the enzymes which transfer the N-acetylgalactosamine (GalpNAc), Galp, and Neup5Ac residues (blue arrow).

Ganglioside functions in cells

Different gangliosides roles have been described in several studies, and there is evidence that cell function and phenotype are influenced by them[21]. There are events

that can be triggered or inhibited by the interaction of gangliosides with extracellular molecules (sugars and proteins). For example, cell growth, differentiation, migration, adhesion, immune interactions, apoptosis, and even tumor aggressiveness and metastasis[2].

Gangliosides fundamental role has been studied on knockout mice and it could reveal several vital functions, particularly in early stages of embryonic development. Studies made in mouse embryos where the glucosylceramide synthase gene was silenced, the resulting individuals have a low chance to survive more than 7.5 days. On the other hand, the ones that have a GM3 synthase knock-down showed a higher sensitivity to insulin and similar effects occur in a GM2 / GD2 synthase knock-down resulting in a lower capacity to repair nervous tissue[2].

Principal gangliosides in cancer cells

Currently, it is well established that the principal difference between a normal and a tumoral cell is the pattern and structure of the carbohydrates that are expressed on their cellular membrane. One of the most evident differences could be found on the carbohydrates associated with ceramide such as glycosphingolipids[3]. Specifically, gangliosides, besides its importance in different cellular processes, they also play an important role in pathological events. Many gangliosides that are present in tumor cells are also found in healthy tissues but the pathogenicity occurs when there is an over-expression on the cell surface, producing malignant transformations due to changes in carbohydrate expression patterns. There is also evidence suggesting that some gangliosides modulate aggressive angiogenesis and tumor growth[22].

Gangliosides are known to be suppressors of the cellular or humoral immune response, and some of them are reported to decrease antitumoral immune cells regulation such as T and B lymphocytes, dendritic cells recognition and also natural killer cytotoxic action[2]. Otherwise, some gangliosides can also have an inhibitory effect on tumor growth[23]. It is known that GM3 ganglioside can act as an inhibitor of the growth of tumor cells due to anti-angiogenesis or motility[24].

Another ganglioside such as N-Glycolyl GM3 was found in colon adenocarcinoma and it also has a role in lung cancer[25]. There is a restricted expression of gangliosides like GM3, GD3, and their derivatives 9-O-acetyl-GD3 and 9-O-acetyl-GT3, in normal breast tissues but there is overexpression of 50 % of them in cancer tissue, in the same way, there is an overexpression of 100% of N-Glycolyl-GM3 ganglioside in breast cancer[26]. Four types of gangliosides have been found in human melanoma cells, among them GM3 and GD3 which have been identified as main components and GM2 and GD2 as minor components[27]. The following table resumes the principal gangliosides: GM1, GM3, GD3, GD2, GT3, among others, which are present in colon, breast, melanoma, lung and nervous system cancer (Table 1).

Colon Cancer	Breast Cancer	Melanoma	Lung Cancer	Nervous System Cancer
GM1 **Present in many cell types. Its reduction increases metastasis.**	**GD3** Present in normal cells. Important in apoptosis. Invasion and growth of the cancer cell.	**N-acetylated GM3** Present in metastatic tumor cells. Promotes metastasis	**GD2** Present in small cells lung. Its reduction decrease cell proliferation and tumor growth.	**GD2** Absent in normal tissue. A major component in the neuroblastoma cells.
NeuGc-GM3 **Present in tumor cells.** **Inhibition of human dendritic cells.** **Act as a potent immune suppressor**	**GD2** Present in cancer stem cells. Its reduction, decrease mammosphere formation, cell motility changing phenotype from CSC to non-CSC.	**GD3** Present in normal cells. Proliferation and motility of cancer cells. Suppress natural killer cell cytotoxicity.	**GD3** Present in normal cells. Invasion and growth of the cancer cell.	**GD1b, GT1b, and GQ1b** Tumor cell proliferation, host immune function, and signal transduction mechanisms. Their expression is linked to the clinical and biological behavior of neuroblastoma tumors.
GD1a **Present in many cell types.** **Metastasis regulator.** **High expression in apoptotic cancerous colon cells.**	**GT3** Present in many cell types. Present in the fetal brain cells.	**GQ1, GD1b, and GT1b** Present in any type of cells. Tumor growth suppressors Interleukin 8 inhibitors.	**GT1b** Present in many cell types. Early apoptosis inducer.	

Table 1: Summary of the principal Gangliosides present in a different type of cancer.

Ganglioside therapy techniques

Even though gangliosides are overexpressed in oncogenic processes, they generate a low immune response, mainly thymus independent, since they are of saccharide origin and self-antigen nature. For this reason, different strategies are needed to be used in immunotherapies. Examples of those are the use of monoclonal antibodies, adjuvants and/or liposomes[11,13,16,28].

NeuGcGM3/VSSP/Montanide ISA 51 vaccine.

NeuGcGM3 gangliosides in combination with a complex of outer membrane proteins of the gram-negative bacteria Neisseria meningitidis that form very small size proteoliposomes (VSSP) and Montanide ISA 51 as an adjuvant are the components of a vaccine (NeuGcGM3 / VSSP / Montanide ISA 51) that has been used in immunotherapies against breast cancer. In phase I of a clinical trial, this vaccine induced a strong immune humoral response in patients with stage III and IV breast cancer. It was reported that after immunization, in 100% of patients with stage III breast cancer, a high response of anti-NeuGcGM3 IgM was shown. Also, in the case of IgG, It was induced in 90% of patients. In addition, it was also shown that the toxicity of the vaccine NeuGcGM3 / VSSP / Montanide ISA 51 was within the limits established by the World Health Organization (WHO)[11].

On the other hand, a phase III clinical trial study showed similar results in the adjuvant setting in patients in stage I and II of breast cancer. Since there was a significant increase in the concentration of anti-NeuGcGM3 IgM and IgG after immunization in both groups of patients (stage I and II). In addition, the majority of patients' hyperimmune sera, not only they could recognize the tumor cells that expressed the NeuGcGM3 ganglioside, but they also had cytotoxic activity on these cells (they killed the cells)[13].

1E10 antibody: active immunotherapy treatment.

The use of anti-idiotypic antibodies against gangliosides present in malignant cell membranes has been recently studied. NeuGcGM3 is an attractive ganglioside which is highly expressed in tumor cells and is undetectable in healthy human tissues[29]. This ganglioside is expressed on breast cancer cells[12] and in advanced lung cancer cells[30]. For that, this ganglioside is considered as a blank for immunotherapy. One approach for producing a long-lasting immune response against GM3 is the use of anti-idiotype antibodies as a way to mimic it. In studies with animal models, BALB/c mice were used, which were immunized with liposomes containing GM3. Then, monoclonal antibodies called P3 (mAb) were obtained. These IgM type antibodies are capable of efficiently recognizing the ganglioside present in tumor cells. Subsequently, immunization of BALB / c mice with mAb P3 was carried out together with Keyhole limpet hemocyanin (KLH) and in this way, it was obtained the 1E10 (mAb)[14].

Racotumomab is the name of 1E10 mAb which is used in the anti-idiotypic vaccines that are conjugated with alumina. During the development of this anti-idiotypic vaccine, specifically on phase I, the principal goals was to identify the dosage, the immunity, and the toxicity[12]. The results suggest that the vaccine was well tolerated and immunologically active[28]. The first fact is appraised because the adverse effects were classified as grade I and II for the National Center Institute Toxicity Criteria (NCIC)[12], whereas the last one is supported because the vaccine displays antibodies specific response against monoclonal antibody 1E10[28]. Despite these facts, anti-idiotypic vaccination could provide survival advantages[12].

In particular, patients immunized with Racotumomab has demonstrated low toxicity and high immunogenicity to this anti-idiotype vaccine showing a strong antitumor and antimetastatic effect in syngeneic and allogeneic models which evidence that it is able not only to mimic GM3(Neu5Gc) but also to activate idiotypically cells that secrete antibodies against this antigens[14].

Valproic acid: chemical treatment.

The degree of GM3 ganglioside expression and the type of tumor cells can produce different effects. In astrocytoma, neuroblastoma, sarcoma, thyroid carcinoma and cutaneous melanoma with high levels of gangliosides suggest aggressive behavior where patients had lower survival. On the other hand, in oral mucosa melanoma, the expression of GM3 (Neu5Gc) was associated with lower risk recurrence and a better prognosis[14]. Additionally, a larger amount of GM3 and a greater amount of GM2 gangliosides control the metastatic cell line, producing a low metastatic cell line in the first case and a high metastatic cell line in the second case[31].

GM3 ganglioside (II3NeuAcLacCer) inhibits epidermal growth factor (EGF) dependent receptor autophosphorylation and cell growth, whereas de-N-acetyl-GM3 (deNAcGM3; II3NeuNH2Lac-Cer) promotes these processes[32]. Epidermal growth factor receptor (EGFR) at membrane microdomains plays an essential role in the growth control of epidermal cells, including cancer cells derived therefrom[33]. GM3 can bind to the receptor by two ways: the union of GM3 and basic amino acid residues of the receptor as it has been found in the case of insulin receptor or GM3 binding via carbohydrate-carbohydrate interaction as it has been observed between GM3 and N-glycans of EGFR[23].

Valproic acid, a histone deacetylase (HDAC) inhibitor, is used as an antiepileptic drug and mood stabilizer, this acid has been used in clinical patients since 1967[15]. Epileptic patients receiving VPA have significantly improved hemoglobin F levels, supporting the hypothesis that non-toxic levels of VPA can induce cell differentiation[15]. It was conducted a study using cancer cell lines founding that VPA stimulates the expression of ST3GAL5 gene that encodes GM3 synthase, the increase in GM3 synthase expression causes an increase in GM3 levels on the cell surface resulting in an inhibition of EGFR phorlation[23]. Consequently, the growth signal is reduced, and cell proliferation is inhibited. The expression of the ganglioside will depend on the level of induction of its synthase gene. It was reported that the treatment of gliomas with valproic acid and radiochemotherapy were well tolerated in children with encouraging response rates[15]. In preclinical studies, VPA inhibited the growth of glial tumor cells in humans and rodents and induced a different mature glial phenotype. In the same way using valproic acid in human tissue with cancer cells (human retinal pigment epithelial cells, carcinoma, and neuroblastoma), resulted in the increase of GM3 expression and reduction of cancer cells proliferation[23,34,35]. Evidence has also been presented that histone deacetylase HDAC inhibitors can sensitize malignant cells to radiotherapy and chemotherapy[15].

14F7 antibody: passive immunotherapy treatment.

One alternative for an active treatment is the use of immunohistochemistry targeting of GM3. The absence of GM3 ganglioside in normal human cells[36] and the presence of the GM3-which is highly specific in cancer cells in different types such as breast[37] , brain[38], melanoma[37], digestive system[36], lung[39], and bladder[40] are the reasons why NeuGcGM3 is one of the most useful and interesting targets for cancer cells in passive immunotherapy[36]. The treatment uses 14F7 which is an antiganglioside monoclonal antibody (mA14F7) of mice. 14F7 mA is specifically defined as a murine immunoglobulin G1 able to recognize the NeuGcGM3[41]. In other words, 14F7 mAb is produced by immunizations of mice of GM3 with human lipoproteins, so it has a specific variable region of isotype and it does not react with other gangliosides[37].

It was demonstrated in vitro with experimental tissue that 14F7 mAb is able to recognize the majority of cancer cells by the presence of GM3 ganglioside in a different

[42,43]

type of cancers: digestive system and lung cancer . The 14F7 mAb binds to the NeuGcGM3 at the cancer cells and through reactions and complement arrangements killing these cells[41]. The antibody binding has a cytotoxic effect[44], the loss of membrane integrity and cytoskeleton constitution changes, which conduce to cell death. First, most of the fragments (fab regions) are recognized by the antigen even when they were compared to another P3 antibody, it was shown to have a high binding capacity and cytotoxicity. Second, the membrane constitution suffers swelling and change in diameter. Third, the activation of FAS receptors and caspases produce cell apoptosis.

BMS-345541 inhibitor of GD2.

A typical neuroectodermal antigen that can be expressed and has relevance in a variety of cancers is the disialoganglioside GD2; this b-series ganglioside can be found on small cell lung cancer, glial tumors, and different sarcomas (bones, bladder)[45–47]. However, it is commonly expressed in neuroectodermal tumors such as skin and uveal melanoma. The study of GD2 is important due to the fact that it is related to cell-to-cell adhesion and in signal transduction. When there is pathology like cancer, this molecule will be involved in processes such as proliferation, neoangiogenesis, immune-scape, and invasion[46].

Different studies have demonstrated that GD2 ganglioside can be found in metaplastic carcinoma samples and in stem cells in breast cancer[46,48]. At this point, it is important to know that GD2 is obtained by the precursor GD3, this molecule comes from the synthesis of the GD3S (GD3 synthase) a highly expressed enzyme on breast cancer cells that has disialoganglioside ($GD2^+$ cells)[47,48].

Orsi et.al in 2017 identified that the inhibition of GD3S on breast cancer stem cells prevents metastasis on *in-vivo* mouse models[46]. This conclusion was achieved due to a deeper analysis of GD2+ cells that shows that they have active signaling of the transcription factor NF-kB. However, when the action of NF-kB is impeded by the inhibitor BMS-345541, the important consequences are the reduction of GD2+ cells and the inhibition of GD3S expression on breast cancer stem cells and cell migration. Those facts contribute to establishing that the inhibitor BMS-345541 suppresses the tumorigenic function of breast cancer stem cells[48].

Treatment Precursor	Treatment Or Vaccine Description	Ganglioside Target	Type Of Cancer	Clinical Phase
BMS-345541 inhibitor of GD2	Inhibit NF-kB by BMS-345541 results in inhibition of GD3S which implies GD2+ cells reduction and therefore its cell migration.	GD2 and GD3 Gangliosides	Breast cancer	Pre-clinical phase
NeuGcGM3/VSSP /Montanide ISA 51 vaccine	The complex of outer membrane proteins of the gram-negative bacteria Neisseria meningitidis and Montanide ISA 51 as an adjuvant.	NeuGcGM3 ganglioside	Breast cancer	Phase I clinical trial Phase III clinical trial
14F7 antibody	Murine antiganglioside monoclonal antibody. Passive Immunotherapy.	NeuGcGM3 Ganglioside	Breast cancer Digestive system tumor cells Lung cancer Neuroblastoma	Pre-clinical phase
1E10 antibody	Anti-idiotypic vaccine. Conjugation of 1E10 monoclonal antibody with alumina. Active immunotherapy	NeuGcGM3 Ganglioside	Breast Cancer Lung cancer	Phase III clinical trial
Valproic acid (VPA)	Stimulates the expression of ST3GAL5 gene that encodes GM3 synthase which inhibits EGFR phosphorylation.	GM3 ganglioside (II3NeuAcLacCer)	Glial tumor cells in humans and mice Neuroblastoma	Preclinical phase

Table 2: Cancer treatment based on gangliosides.

CONCLUSIONS

Gangliosides research as target molecules in immunotherapies and treatments for cancer has been developed since the important, evident, and useful aspects of the role of gangliosides in the regulation of cell proliferative signals. Different immunotherapies and treatments with gangliosides have been developed. Examples of that are BMS-345541 inhibitor of GD2, 14F7 antibody, and valproic acid which are still on the preclinical phase; whereas NeuGcGM3/VSSP/Montanide ISA 51 vaccine and 1E10 antibody have already reached phase III on clinical trials. These achievements are important because it shows the potential application of gangliosides as targets to deal with cancer cells. By this way, recognizing the gangliosides that are present on certain cell lineage will allow developing new biomarkers, antibodies or metabolic pathways that could enhance the immune response in a specific and less aggressive way. Therefore, this fact could increase the survival rates of cancer patients.

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