Review Article

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A Review of the Role of Sphingolipids in Apoptosis Phenomenon

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ABSTRACT

BACKGROUND AND OBJECTIVE: Cancer is one of the major health problems in the world and chemotherapy is still the most common solution for its treatment. A great deal of studies in this area have been devoted to evaluating the occurrence of apoptosis as a key factor in preventing cell's escape from cell cycle regulation mechanisms. The aim of this study is to summarize the studies on metabolism, messenger pathways and effective pharmaceutical factors on sphingolipids involved in apoptosis.

METHODS: In this review article, the national and international databases of PubMed, Scopus, Google Scholar, Web of Science, ISC and Magiran were searched for the keywords “apoptosis”, “sphingolipids”, “ceramide”, “sphingosine” and “cancer” without time limit and the related material was collected.

FINDINGS: Among the apoptotic messenger molecules, the key role of the sphingosine and ceramide has been considered as the cornerstone of sphingolipids in many of its controlling processes. It has been shown that ceramide is a key regulator in apoptosis, and increase in its cytoplasmic levels increase the proliferation of cascades resulting in programmed cell death. The bio-production and bio-destruction of ceramide is accomplished by the activity of several enzymes, and much evidence suggests the effect of external factors on enzyme systems. In contrast, the phosphorylated form of sphingosine is an important index for guiding cells toward proliferation and differentiation. It has been found that several commonly used chemotherapy drugs and compounds that are being studied in the treatment of cancer affect at least one of the enzymes of sphingolipids metabolism.

CONCLUSION: Sphingolipids and the enzymes involved in their metabolism are introduced as new pharmacological targets for the induction of apoptosis, and it is obvious that analyzing the effective therapeutic factors and the ways of controlling them would be helpful in finding anticancer drugs.

KEY WORDS: *Apoptosis, Sphingolipids, Ceramide, Sphingosine, Cancer.*

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Introduction

In the past, lipids were mainly considered in molecular cell research for their role in maintaining the structure of the cell membrane and protecting it from environmental stresses. The findings of the past two decades have greatly changed this concept, and today lipids play a key role as second messengers in regulating various cellular processes, including inflammation and fever, survival, cell proliferation and division, differentiation, aging and programmed cell death (1). Meanwhile, sphingolipids are of great importance as lipid second messengers, and the factors affecting them have been the subject of various studies (2). Sphingolipids are a group of lipids based on alcohol sphingosine that are produced in endoplasmic system (1). Changes in the root of sphingosine caused by various enzymatic systems eventually lead to the formation of sphingolipids with a structural role in the cytoplasmic membrane as well as bioactive mediators in the regulation of cellular homeostasis (3). The occurrence of apoptosis or programmed cell death, as one of the most important processes for maintaining the homeostasis of multicellular organisms, is generally used to remove unwanted or unnecessary cells and it interferes with various mechanisms of immune system or diseases (4). Apoptosis plays a vital role in various important biological processes, such as natural development, and the removal of defective, and infected cell and activated immune cells against self-antigens. The occurrence of many autoimmune diseases, cancers and infections is the result of poor performance, inhibition, or out of control process of this phenomenon (5). The loss of the balance in the speed and rate of occurrence of this phenomenon, either decreasingly or increasingly, leads to cancer or neurological disorders such as Alzheimer's disease and Parkinson's disease, respectively (4, 5). Considering the importance of protective processes of cells against apoptosis as well as its inducing factors and ultimately the balance between these factors, evaluation of the stimulating or inhibiting factors and messenger pathways in the occurrence of apoptosis has been considered as one of the most appealing and useful subjects in different fields of biological sciences. Among the regulating factors, this physiological (and sometimes pathologic) process of some sphingolipid molecules showed a determining role. It has been shown that the phosphorylated form of sphingosine is one of the most potent inhibitors of the occurrence of apoptosis and mitogenic stimulation. In addition, the phosphorylated form of ceramide, as the precursor of sphingosine, is one of the most effective inducers of apoptosis (1, 6). In addition, it has been shown that the balance between sphingosine and ceramide is a determining factor for cell's fate and it uses various functions to send a message of life or death (3). In recent years, the enhancing or inhibiting factors of such functions as well as the important enzymes in the metabolism of sphingolipids have been the subject of discussion in various cell studies as novel pharmacological targets in the induction or inhibition of apoptosis and other cellular processes related to these messengers (7). Given the extensiveness and complexity of enzymatic systems and ceramide and sphingosine executioners as the most significant sphingolipids that regulate apoptosis and considering the introduction of some drugs that affect these pathways into clinical trials and attempts to enhance their structure-effect relationship, further evaluations on this topic can be the basis for the treatment of various associated disorders, including cancer (3, 8). Therefore, the main aim of the present study is to collect previous research findings and provide a comprehensive overview of their results for the development of new chemotherapy drugs and to get familiar with the mechanisms involved in the antitumor effects in this regard.

Methods

In this review article, the national and international databases of PubMed, Scopus, Google Scholar, Web of Science, ISC and Magiran were searched for the keywords “apoptosis”, “sphingolipids”, “ceramide”, “sphingosine” and “cancer” without time limit.

Results

Apoptosis and its implementing agents: The occurrence or non-occurrence of programmed cell death, like any other physiological phenomenon, is the result of balance between a large number of messenger molecules with anti-apoptotic or pre-apoptotic effects that plays an important role in its regulation (5, 9). By activating a large family of proteases called initiator caspases, these messenger pathways, and operators lead to the activation of executioner caspases, and they ultimately lead to programmed cell death through chromatin condensation and fragmentation (4). Caspases, a group of cysteine proteases, are among the proteolytic enzymes and the induction of initiator caspases results in the breakdown of the pre-caspases of

inactive dimers and their conversion into active and executioner caspases (5). Although the internal (mitochondria-dependent) and external (dependent on death receptors on the cell membranes) pathways have been identified as the main pathways for induction of apoptosis, recently other pathways such as endoplasmic reticulum and lysosomal pathways have also been identified as apoptosis inducers (4, 10). The p53 molecule is also one of the most effective tumor suppressors that play a pivotal role in stimulating the internal pathways of apoptosis and DNA damage is the most important stimulus for its initiation (11).

In the internal pathway, factors such as environmental stress, heat, hypoxia, lack of growth factors, intracellular infections, as well as the caspase 8 activated within the external pathway of apoptosis have led to increase in the expression of pre-apoptotic proteins of B-cell lymphoma family and decrease in anti-apoptotic proteins such as Bcl-2 (5, 9, 12). The sum of these factors leads to an increase in the permeability of the mitochondrial membrane and creates holes on its surface, which results in the release of cytochrome C from the mitochondria into the cytoplasm (13). The release of these agents activates caspase 9 by producing the apoptosome complex and the activated caspase 9 leads to apoptosis by activating the executioner caspases (caspase 3) (14).

In the external pathway, the activation of death receptors of TNF family such as CD95 in the cell membrane results in the binding of these receptors from their intracellular sequences to the corresponding ligands, and ultimately produce mediators such as Fas-associated death domain (FADD), which activates caspase 8 (4). Caspase 8 also causes apoptosis by increasing the expression of the pre-apoptotic proteins of the B-cell family and by activating executioner caspases (9).

In the lysosomal pathway of apoptosis, the release of lysosomal proteolytic enzymes such as cathepsin B, acid sphingomyelinase, acid esterase, etc., will eventually cause apoptosis (10, 15), regardless of being able to move the cell towards autophagy. The endoplasmic reticulum is an important organelle in regulating the phenomenon of apoptosis, which plays a major role in this biological process by developing a series of apoptosis or anti-apoptosis stimulants, including Bcl family proteins, death receptor ligands, and the release of calcium into the cytoplasm (2). In addition, de novo ceramide synthesis, which is one of the most important phospholipid-based second messengers in programmed cell death, occurs in the endoplasmic reticulum. Golgi apparatus also plays a major role in protecting cells from the apoptosis process by developing glucosylceramides and P-glycoproteins (1).

The structure and role of sphingolipids in cellular physiology: Sphingolipids are lipids with a complex sphingosine nucleus that, along with glycerolipids and sterols, constitute the major part of cell membrane structure. Sphingosine is the foundation of the structure of the sphingolipids of cell membrane, which is N-acetylated by 6 – 24 carbon fatty acids and forms the ceramide molecule (16). Quantitatively speaking, ceramides are one of the most important components of cell membranes and play a special role in protecting cells from environmental stress (1). The addition of phosphoethanolamine, monosaccharide, and oligosaccharide along with sialic acid to ceramide leads to the formation of sphingomyelins, cerebrosides and gangliosides, which play role as biological markers, neuronal membrane insulators and extracellular ligand-binding agents, in addition to their structural role (3, 17). However, it has been shown in the last two decades that sphingolipids, especially simpler sphingolipids, i.e. ceramides and sphingosines, in their phosphorylated form as messenger molecules, are influential factors in many physiological and pathological processes of cells, and the quantitative balance between them will determine the fate of the cell (1).

These messenger molecules also affect the regulation of the processes of proliferation, cell division and differentiation, fever, inflammation, aging, glucose and lipid metabolism, and many other factors. In contrast, sphingolipids that are more complicated play structural or marker role and except for a few cases, they do not act as second messenger (18).

Sphingolipids, messenger molecules in cell life cycle: The results of numerous studies in recent years have indicated the determining role of total cellular sphingosine and ceramide levels (in phosphorylated form) in the formation of cell death or survival message. If this balance shifts toward increase in ceramide levels in any way, various pathways of apoptosis, including the internal, external, and joint pathways are activated quickly and the cell is directed toward apoptosis in a short time (2). Conversely, the breakdown of ceramides in the cytoplasm leads to an increase in the levels of intracellular sphingosine by increasing the activity of various isoforms of ceramidase enzyme (19). By mediation of environmental growth factors and cytokine-induced proliferation, the mediator produces the phosphorylated form of sphingosine, and this active sphingolipid is a potent stimulant of the factors involved

in cell division, including phosphoinositide 3-kinase (PI3K) and phospholipase C (6). Figure 1 shows a summary of the messaging pathways of ceramide and sphingosine in determining the fate of the cell.

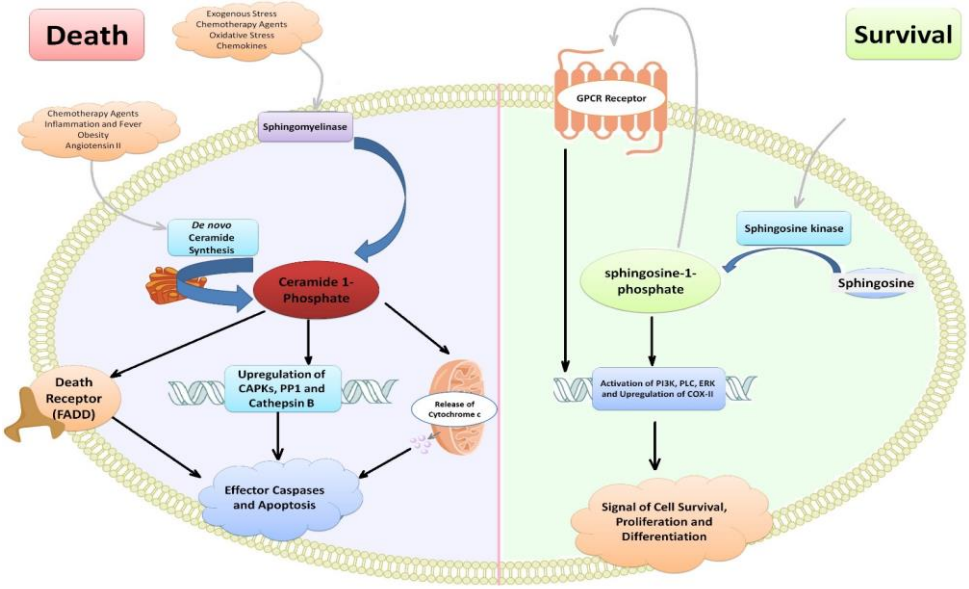

Figure 1. The balance between total cellular phosphorylated sphingosine and ceramide levels as regulating factors of cell death or survival message (1, 2, 6)

By increasing the total cellular ceramide levels, the activation of the major pathways of ceramide production stimulates the release of cytochrome C (Cyt C), affects the downstream pathways, activates death receptors or FADD, and most importantly, increases the expression of ceramide of executioner caspases and induces apoptosis. Increase in the destruction of ceramide and its transformation into sphingosine and the activity of sphingosine kinase lead to an increase in the levels of intracellular phosphorylated sphingosine. This mediation promotes the survival and proliferation messages from the genome, both through its G protein receptors, and by activating and increasing the expression of PI3K, phospholipase C, extracellular signal-regulated kinase (ERK) and cyclooxygenase – 2 (COX – 2) directly.

The addition of external ceramide also induces programmed cell death, and recently, ceramide nanoparticles have shown promising effects in the treatment of chemotherapy-resistant tumors (7). The importance of ceramide pathway in the induction of apoptosis is to the extent that ceramide has been introduced as the key to apoptosis (21).

The main pathways of sphingolipids metabolism as new chemotherapy objectives: Considering the importance of messenger ceramide pathway in inducing apoptosis in tumor cells, the factors that strengthen the external ceramide levels, its executioners and the factors that influence its metabolism have been the subject of numerous studies over the past decade (7). Therefore, familiarity with the enzymatic systems involved in the metabolism of ceramide and sphingosine as significant sphingolipids in cell survival and cell death, as well as the executioners and their mechanism of action can be very useful in this regard (17). Many inhibitors of ceramide destruction and many of its construction reinforcers are under clinical phases to be used as chemotherapy drugs for tumor (22). In addition, the production of ceramide and enhancement of its executioners are known as the second antitumor mechanism of action for many common chemotherapy and radiotherapy factors (19). Figure 2 shows a summary of various pathways of intracellular ceramide metabolism.

De Novo pathway: The onset of cytoplasmic formation of ceramide and the major part of the sphingolipids, also known as De Novo ceramide synthesis, is a part of endoplasmic reticulum and ceramide is made from Palmitoyl-CoA and serine in five steps (17). The first and the most important step of ceramide formation, which is also the rate-limiting step, is the concentration of amino acid serine and Coenzyme A + Palmitoleic acid, which is catalyzed by serine palmitoyltransferase (SPT) and results in 3-ketosphinganine (1).

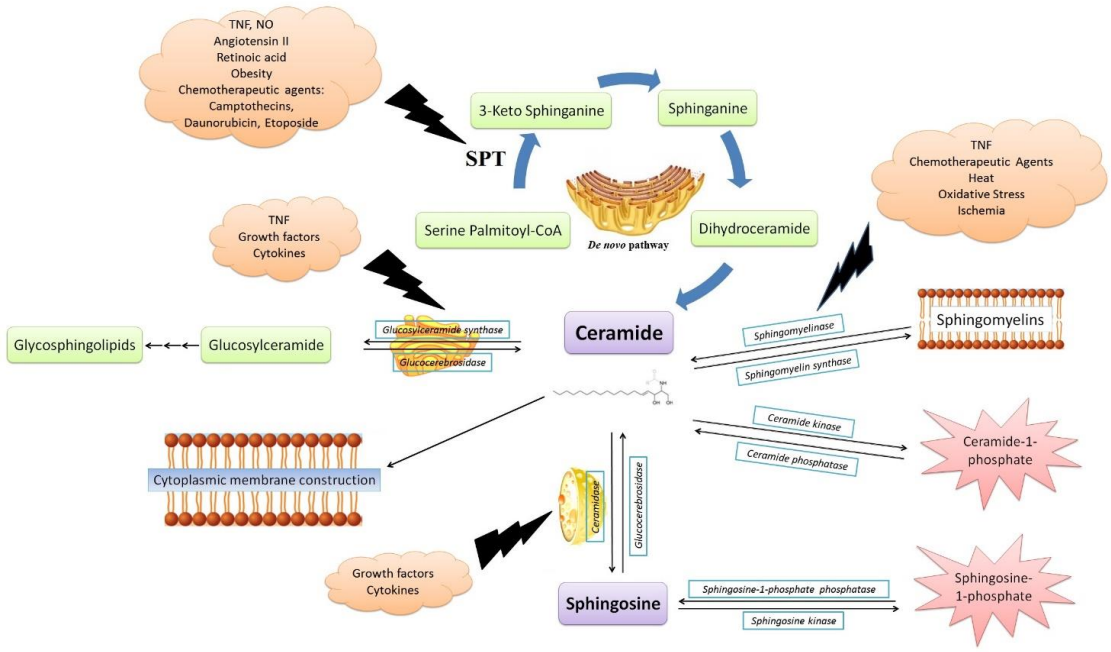

Figure 2. Main pathways of ceramide metabolism (3, 7, 17)

Then, 3-ketosphinganine is restored, N-acetylated and desaturated, and eventually, after the addition of Acyl-CoA with different numbers of carbon atoms, ceramide is formed with an acyl group with 6-22 carbon atoms (17). It has been shown that inhibition of ceramide formation through suppressing this pathway by SPT-specific inhibitors such as Fumonisin II has led to a reduction in the response to tumor chemotherapy and, conversely, a mutation in this enzyme and its high basic activity lead to inherent defect of sensory nerves (23). Various biological factors such as nitric oxide, TNF, angiotensin II, and the abundance of palmitoleic acid reserves, conditions such as obesity, as well as the presence of pharmacological factors such as retinoic acid derivatives, camptothecin, daunorubicin and etoposide stimulate the activity of SPT (1, 17).

Sphingomyelinase pathway: Ceramides formed in the endoplasmic reticulum are introduced into the Golgi apparatus by special carriers, are subjected to different isoforms of the sphingomyelin synthase enzyme, and are converted to sphingomyelin with the same number of carbon atoms as the original ceramide by conversion of phosphorylcholine to diacylglycerol (31). The product of this reaction is mainly used for the production of myelin of neural cell membrane or conversion to ceramide near one of the isoforms of sphingomyelinase (32). The acidic form of sphingomyelinase is one of the most important isoforms of this lysosomal enzyme that exits during metamorphosis of the lysosomal membrane and causes degradation and conversion of sphingomyelin to ceramide by reaching the cytoplasmic membrane (26). Therefore, its reinforcement is an objective for anti-tumor drugs. Neutral and basic isoforms of sphingomyelinase are mainly present in the cytoplasm and are often magnesium- and zinc-dependent, and the abundance of these heavy metals is considered as reinforcing factors for these enzymatic systems (33). The suppression of this pathway in cancer cells has been introduced as a factor for resistance to some chemotherapy drugs (25, 26).

The enzymatic systems that convert sphingomyelin to ceramide include various biological responses to inhibit and reinforce them. Factors such as interferon gamma, interleukin-1, heat, oxidative stress, ischemia / reperfusion stress, and factors associated with neutral sphingomyelinase as biological factors and drugs such as vitamin D3, doxorubicin, mitoxantrone and silibinin have been suggested as pharmacological agents that reinforce sphingomyelinase (24, 30).

These drugs either have anti-tumor effects or are likely to have tumor-suppressive effects by increasing the formation of ceramide through the induction of sphingomyelinase. On the other hand, according to some studies, sphingomyelinase inhibitors such as desipramine and amitriptyline show protective effects against apoptosis in some cells, including the liver cells (26).

Ceramidase pathway: The ceramide present in the cytoplasm can be broken down in the same spot or in the lysosome under the influence of various isoforms of ceramidase enzyme and be converted to sphingosine

and its constituent fatty acid. Therefore, not only does the cell survive the death that is mediated by ceramide, but also its sphingosine becomes activated and enters the phase of cell division (34). Consequently, inhibitors of this enzyme can be considered as tumor suppressor agents (35). Among the three acidic, basic, and neutral isoforms of the ceramidase enzyme, the acidic form that is present in the lysosome has been reported as the most important form in various studies (19). Biologic agents such as TNF, IL-1, and platelet-derived growth factors, and pharmacological agents such as tamoxifen and dacarbazine are known as ceramidase inhibitors and apoptosis inducers with ceramide accumulation from this pathway. The mutation in this enzymatic system and its lack of response to dacarbazine is a factor for the resistance of the tumor cells against this drug (35). It has been shown that menthol and limonene are able to accumulate by inhibiting this enzyme and induce a ceramide-dependent programmed death in colon cancer cell line (36). The significance of ceramidase in the biology of cancer is in such a way that its selective inhibitors are developing and some of them, such as DMAP and B-13, enter the clinical phases for the treatment of some tumors, such as the malignant tumors of the prostate (22, 37). Considering the presence of the most important isoform of the ceramidase enzyme, i.e. acid ceramidase, in lysosome, gaining access to it and changing the structure-effect relationship of drugs in order to increase their efficacy have been one of the challenging issues in the studies in this area (8, 28).

Glucosylceramide synthase (GCS) pathway: The entrance of ceramide into Golgi apparatus is a gateway for the formation of glycosphingolipid. In this case, the first step is the addition of glucose from UDP-glucose to ceramide by the glucosylceramide synthase (GCS) enzyme and the formation of glucosylceramide (17). In the next step, various products of glucosylceramide, which mainly play structural role and act as cell markers, are derived from this mediator, or additional glycosylated ceramides in the opposite pathway of the above reaction are converted to their constituent ceramide by the cerebrosidase enzyme. Thus, the activity of CGS saves the cell from ceramide-induced apoptosis and is a way to resist chemotherapy (38, 39). However, the role of inhibitors of this pathway in the elimination of ceramide was not significant in studies about programmed cell death, and GCS inhibitors such as GZ667161 were shown to be effective in some disorders of the nervous system (29). In previous studies, it has been shown that some of the derivatives of silibinin, hesperidin, and rosmarinic acid cause ceramide accumulation in cancer cells by inhibiting this enzyme (30, 40, 41).

The most important pathway for ceramide formation is its De Novo synthesis, in which the concentration of amino acid serine and Coenzyme A + Palmitoleic acid occurs by the SPT enzyme. Then, ceramide is finally obtained in three other enzymatic steps. The resulting ceramide can be converted to sphingomyelin, or the sphingomyelinase converts the sphingomyelin in the cytoplasmic membrane to ceramide. GCS present in the Golgi apparatus can glycosylate the ceramide present in the cytoplasm to eventually produce glycosphingolipid. Glucocerebrosidase is another enzyme that unlike the previous pathway rearranges ceramide. Ceramide breakdown by various isoforms of ceramidase acid and its conversion into sphingosine and its constituent fatty acid is another event that affects ceramide. The ceramide synthase enzyme acts opposite to the previous reaction and reproduces ceramide by binding the sphingosine to the fatty acid. The phosphorylation of ceramide and sphingosine makes them the active forms of biological messengers. Free ceramide in the cytoplasm can be used to produce cytoplasmic membrane.

Ceramide and its messaging pathways: Phosphorylated ceramides use several executioners to make the order of programmed cell death. In fact, the messaging pathway of ceramide is upstream of many other messenger molecules, and it is important in this regard (21). Of course, all three pathways of apoptosis are directly stimulated by ceramide pathway, though ceramide executioners seem to play a more significant role in terms of multiplicity and efficiency (2, 43). The executioners of ceramide's messaging pathway are divided into three categories of kinases, phosphatases, and proteases (43).

Sphingosine and its messaging pathways: Sphingosine resulting from the breakdown of ceramides is phosphorylated by the sphingosine – kinase – 1 enzyme through stimulating external factors such as growth stimulants and some cytokines, as well as the downstream activation of the TNF receptor, and acts as a bioactive mediator. The phosphorylated sphingosine organizes and guides the cell towards division and differentiation by influencing some of the regulatory factors in the cytoplasm or by effecting the receptors of its G-protein on the membrane surface (mainly opposite to the ceramide pathway) (6). Thus, higher expression of sphingosine – kinase–1 in cells with high

proliferation and division, such as many of the cells in the immune system, mucous membranes, etc., would be unexpected (1).

The key role of the sphingosine's messaging pathway and its receptors has been identified in many biological phenomena such as angiogenesis, vascular sustainability and permeability, traffic of B and T lymphocytes and sometimes, immunosuppression (6). Phosphorylated sphingosine directly inhibits apoptosis and induces cell division by directly activating COX II, ERK and NF-κB and disabling caspase 3. The mediator can also exit the cell and affect its G-protein receptor through autocrine or paracrine signaling, and activate various messaging pathways depending on the type of receptor. Different types of receptors of this family including G_{12}/G_{13}, G_q / G_1, G_s and G_i / G_o are introduced as sphingosine – kinase–1 messengers. The multiplicity and diversity of these receptors justifies some of the contradictory effects observed from sphingosine in cellular life (6).

The role of other sphingolipids in apoptosis: Glycosphingolipids with sugar-complex structure mainly play a structural role in the cell membrane and apart from that, act as cell markers and toxic receptors of cell. For example, toxin uses some microbial agents from gangliosides as pathogen-binding agents to enter the cell (44). It has been observed that in some areas of the brain during development, ganglioside expresses GM3 and GD3 significantly more than other regions, which indicates their potential role in the development of the nervous system (45,46). While monoclonal antibodies against ganglioside GD2 have beneficial effects in the treatment of melanoma, it has been shown that GD3 is one of the downstream products of ceramide glycosylation, induces apoptosis, and plays an important role in the elimination of unwanted cells during development (39, 47). Although increased levels of GM3 associated with Parkinson's disease are of unknown origin, some studies have shown that this factor protects and increases neuroplasticity by imitating neurotrophic factors, and its derivatives have been useful in the treatment of Alzheimer's disease (44, 48, 49).

Discussion

The occurrence of apoptosis is one of the main protective methods for multicellular organisms in eliminating excess cells or avoiding cell cycle regulation processes and preventing them from entering the neoplasm phase (4). The balance of intracytoplasmic levels of sphingosine / ceramide as the most significant apoptosis-regulator sphingolipids determines the fate of the cell, and drug interventions on the reinforcing or facilitating factors of each of them will promote the movement of the cell towards death or survival (50, 51). Ceramide synthesis stimulants, including the stimulation of death receptors, nutrient deprivation, hypoxia and chemotherapy agents, increase the levels of total ceramide and cell death, and the regulation of ceramide levels in cancer cells has been proposed as an effective method for inducing their deaths (1, 43). The addition of ceramide of external origin and inhibition of its destructive enzymes are two essential strategies for the elimination of cancer cells, which could be the subject of discussion for future studies (52). Increase in the knowledge of molecular biology in the context of metabolizing enzymatic systems, dependent downstream intracellular executioners, as well as their external effective factors will open new horizons in the treatment of diseases caused by apoptotic disorders such as cancer, diabetes, Parkinson's disease, Alzheimer's disease, etc. (53). In addition, the vital role of lipids, and in particular ceramide, in controlling the cell cycle provides a new perception about the biology of cancer (54). Therefore, sphingolipids and enzymes involved in their metabolism are introduced as new pharmacological targets for induction of apoptosis. Obviously, the study of effective therapeutic factors and their ways of controlling cancer would help us find novel anticancer drugs.

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