ISSN 0006-2979, Biochemistry (Moscow), 2006, Vol. 71, No. 1, pp. 10-17. © Pleiades Publishing, Inc., 2006.
Original Russian Text © E. V. Dyatlovitskaya, A. G. Kandyba, 2006, published in Biokhimiya, 2006, Vol. 71, No. 1, pp. 17-25.

REVIEW

Role of Biologically Active Sphingolipids in Tumor Growth

E. V. Dyatlovitskaya* and A. G. Kandyba

Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, 117997 Moscow, Russia; E-mail: dyatl@ibch.ru

Received February 10, 2005
Revision received March 17, 2005

Abstract—This review highlights the literature on the effects of biologically active sphingolipids (sphingosine, ceramide, sphingomyelin, glucosylceramide, gangliosides GM1, GM2, GM3, GD3, etc.) on proliferation, apoptosis, metastases, and invasiveness of tumor cells and the putative role of sphingolipids in chemotherapy of malignant tumors.

DOI: 10.1134/S0006297906010020

Key words: gangliosides, glucosylceramide, sphingosine, sphingolipids, sphingomyelin, sphingosine-1-phosphate, ceramide, tumor

Sphingolipids represent the most chemically diverse class of lipids, which exhibit a wide spectrum of biological activity. All members of this class contain one common structural element, a sphingosine base. Sphingosine is the trivial name of C_{18}-sphingenine (D-*erythro*-(2S,3R,4E)-2-amino-4-octadecene-1,3-diol), the most common sphingoid widely distributed in human and animal cells. The scheme below shows metabolic links between all cellular sphingolipids.

In 1986, it was demonstrated that free sphingosine (Sph) inhibits protein kinase C *in vitro* [1]. After that several thousand papers appeared on the involvement of various sphingolipids in cell proliferation, differentiation, and apoptosis and also in some other biological processes.

Good evidence now exists that such sphingolipids as sphingosine [2] and ceramides, N-acylsphingosines (see reviews [3, 4], for example) inhibit cell proliferation and stimulate apoptosis, whereas their metabolites, sphingosine-1-phosphate (S1P) [4-7], sphingosine-1-phosphocholine [4, 8-10], and ceramide-1-phosphate [4, 9], stimulate proliferation and inhibit apoptosis. There are contradictory data on the effect of sphingomyelin (SM) on the cell cycle.

Results of numerous studies of the effects of glycosylated ceramides, glycosphingolipids, on cell proliferation and apoptosis revealed that these lipids are actively involved in cell growth, and their action depends on the structure of the carbohydrate chain [7].

Biologically active sphingolipids play an important role in malignant growth. Ten years ago Hannun et al. suggested that Cer are tumor suppressors [11, 12]. Subsequent studies revealed that almost all types of sphingolipids influence tumor growth, and the resultant effect depends on their balance in the cell. Recently possible use of sphingolipids for chemotherapy of malignant tumors has been actively discussed [13-20].

In the present review we consider data on the effects of biologically active sphingolipids on proliferations, apoptosis, metastases, and invasiveness of tumor cells.

Free **sphingosine** is the simplest sphingolipid present in animal and human cells. It was previously shown that at low concentrations (<10 μM) it stimulates DNA synthesis, but higher concentrations cause cell death [21]. Sphingosine also inhibits proliferation [22] and stimulates apoptosis [22-27] of various malignant tumor cells. The anthracycline antibiotic Doxorubicin used for cancer chemotherapy stimulates apoptosis of breast carcinoma MCF-7 cells. This is accompanied by significant increase in sphingosine content, caspase-7 activation, and release of cytochrome *c* from mitochondria. Similar effects have also been observed after treatment of these cells with exogenous sphingosine. This suggests involvement of sphingosine in apoptosis mediated by the mitochondrial pathway [26, 27]. Sphingosine also influences phosphorylation of epidermal growth factor receptor in carcinoma A-431 cells [28].

Ceramides, N-acylated derivatives of sphingosine, are key intermediates of sphingolipid metabolism (Scheme); they also inhibit proliferation and stimulate

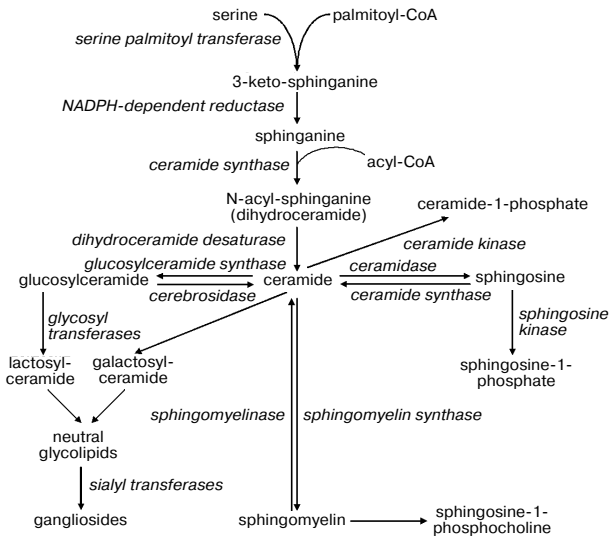
Abbreviations: Cer) ceramide; ERK) extracellular signal regulated kinase; Fas) death receptor initiating apoptosis; GlcCer) glucosylceramide; S1P) sphingosine-1-phosphate; SM) sphingomyelin; Sph) sphingosine; SphK) sphingosine kinase.

* To whom correspondence should be addressed.

apoptosis of cancer cells. This problem has already been considered in the review by Dyatlovitskaya [29]. Here we will analyze recent data on the mechanism underlying the effect of ceramide on tumor growth. Some tumor cells are characterized by lower content of Cer compared with cells of normal tissues [30-32]. Ceramide levels were inversely associated with malignant progression of tumors [30, 31]. However, in many tumors Cer level is higher than in normal tissues [33, 34]. This suggests lack of direct correlation of malignant growth on ceramide content. Good evidence now exists that the effect of ceramide depends on localization of this lipid. Various sphingomyelinases are involved in formation of endogenous Cer. Since acidic sphingomyelinase is mainly localized in lysosomes, and neutral sphingomyelinase is in cytoplasm and plasma membrane, it is clear that their action yield different Cer pools.

Apoptosis of human glioma cells induced by Fas death receptor antibodies was characterized by increased activity of acidic (but not neutral) sphingomyelinase and generation of Cer. Both processes depend on caspase-8 activation [35]. Ceramide generation in human colon cancer HT-29 cells by exogenous neutral and acidic sphingomyelinase triggered different apoptotic pathways [36]: neutral sphingomyelinase preferentially activated complexes of the nuclear factor NF-κB with Rel A/p52- and Rel A/p50-dimers (within 30 min), whereas activation of the complexes of the nuclear factor with p50/p50 dimer by acidic sphingomyelinase required a much longer period (20 h). These HT-29 cells were sensitive to acidic sphingomyelinase and resistant to neutral sphingomyelinase. Some antitumor preparations increased Cer content in the tumor cells due to their effect on *de novo* synthesis rather than stimulation of sphingomyelinase activity [37].

Exogenous Cer also stimulates apoptosis in various tumor cells [22, 38-44]. However, it should be noted that ceramides induce not only apoptosis but also necrosis of cells [45, 46]. Sometimes exogenous Cer inhibited proliferation without induction of apoptosis [47]. In some cases exogenous Cer induced sensitivity of tumor cells to antitumor preparations [38] and γ-irradiation [48]. Detailed study of the mechanism responsible for apoptosis stimulation by ceramide in A-549 lung carcinoma cells revealed that ceramide inhibited phosphorylation of transcription factor c-Jun, but promoted proapoptotic Bim protein phosphorylation and its translocation to mitochondria [43]. In SKN-SH neuroblastoma cells Cer altered the content of p53 regulating the ratio of Bcl-2/Bax and caspase activation during apoptosis [40]. In human HCT-116 colon carcinoma cells Cer stimulated binding of nuclear factor NF-κB with DNA, caspase-3 activation, cytochrome *c* release from mitochondria [42], and also induction of Bax, a protein product of a proapoptotic gene [39]. All these data indicate involvement of the mitochondrial pathway in apoptosis of tumor cells. However, in human glioma cells, Fas-induced apoptosis



Sphingolipid metabolism

Scheme

accompanied by stimulation of acidic sphingomyelinase and accumulation of endogenous Cer depended on activation of caspase-8 but not p53 [35].

Phosphorylation of sphingosine yielding **sphingosine-1-phosphate** (S1P) removed suppressor activity of sphingolipids. S1P formed by sphingosine kinase stimulates proliferation and inhibits apoptosis of tumor cells. In contrast to ceramide, which exhibits properties of second messenger, S1P acts as both second messenger and extracellular modulator [49-53]. Now the whole family of G-protein-coupled receptors, which bind exogenous S1P, has been identified [5, 49-54].

S1P stimulates proliferation and inhibits apoptosis of tumor cells and thus promotes tumor cell survival. In human leukemia Jurkat and U-937 cells, S1P prevented Cer-induced apoptosis by inhibiting mitochondrial caspase-3 and release of mitochondrial cytochrome *c* [55]. S1P also promoted mouse melanocyte survival by activation of Akt and ERK (extracellular signal-activated kinase) [56]. The role of endogenous S1P in tumor growth was also evaluated by changes in sphingosine kinase (SphK) activity. Cells overexpressing this enzyme had increased SphK activity and acquired the transformed phenotype. The authors of this report reasonably consider a gene encoding this enzyme as an oncogene [57]. Survival of PC-12 cells depended on regulation of caspase and stress-activated protein kinase activation by SphK [58]; this enzyme also plays an important role in breast cancer progression [59]. The effects of SphK on tumor growth have been recently reviewed [60].

It is known that S1P is also present in the blood stream where it is produced by platelets [7]. This extracellular S1P can bind to specific cellular G-protein coupled receptors influencing motility of tumor cells, their

metastases, and invasiveness. It was previously shown that S1P inhibited motility of some tumor cells [61-63]. Recently it has been demonstrated that S1P stimulates motility of glioblastoma cells and their invasiveness [64]. Regulation of tumor cell motility and invasiveness depends on the type of S1P receptor [65]: $S1P_1$ receptor mediates stimulation of motility [66, 67], whereas $S1P_2$ receptor causes inhibition of motility and invasiveness [67, 68]. "Cross-communication" of these receptors is also important for the resultant effect [65]. S1P also promotes tumor angiogenesis by stimulating endothelial cell motility [69, 70], and this process can be blocked by receptor antagonists [71].

Since Cer and S1P exert opposite effects on cell processes, the resulting biological effect depends on the ratio of these metabolites in particular cells. Some authors even suggest that this ratio determines cell fate [5, 49, 54]. However, besides S1P another intermediate, **glucosylceramide** (GlcCer), also stimulates proliferation and inhibits apoptosis. This metabolite contains a glucosyl residue at C1 of the sphingoid chain (see Scheme). Recent data suggest that cells sensitive and insensitive to antitumor preparations are characterized by different metabolism of sphingolipids [72] and drug resistance of cells depends on degree of Cer glucosylation [73-75]. Accumulation of GlcCer in tumor cells increased their resistance [76, 77], whereas blockade of Cer conversion to GlcCer increases their sensitivity to antitumor preparations [78, 79]. Degree of Cer glucosylation mainly depends on expression and catalytic activity of glucosylceramide synthase [80]. Blockade of this enzyme activity in leukemic CCRF-CEM cells enhanced vincristine-induced cytotoxicity [81]. Inhibition of glucosylceramide synthase in human MCF-7Adr mammary carcinoma cells resistant to doxorubicin increased sensitivity of these cells to antitumor preparations [82]. In experiments with ME-4 melanoma cells inhibitor of this enzyme not only increased sensitivity of these cells to antitumor preparations but also decreased tumor progression [83]. Introduction of the glucosylceramide synthase gene into human MCF-7 breast cancer cells increased expression of this enzyme, and reduced DNA fragmentation (i.e., reduced degree of apoptosis) and increased resistance of the cells to Cer [84].

However, it should be noted that inhibition of this enzyme not always increases drug sensitivity of cells. Studying melanoma cells characterized by different levels of glucosylceramide synthase, Veldman et al. concluded that certain care should be taken for interpretation of data indicating importance of ceramide glucosylation for drug resistance of tumor cells [85]. Employment of novel glucosylceramide synthase inhibitors in cultured leukemic U-937 and HL-60 cells revealed that these inhibitors protected cells against apoptosis and Cer galactosylation occurred instead of glucosylation [86]. Attempts to use novel glucosylceramide inhibitors to increase the sensitivity of drug resistant NCI/Adr and MES-SA/DX-5 cells were not successful [87].

Sphingomyelin (SM), ceramide-1-phosphocholine, is a sphingolipid inhibiting progression of gastrointestinal tract tumors. SM in food was shown to prevent malignant tumor formation [88]. It also decreases the number of tumor cells [89] and normalizes cell proliferation, and this results in suppression of malignant growth [90]. Alkaline sphingomyelinase cleaving SM in the gut also promoted suppression of tumor growth in an apoptosis-independent manner [91].

There is evidence that SM not only inhibits tumor progression in the gut, but also induces apoptosis in other tumor cells *in vitro* (e.g., in breast cancer MCF-7 cells), and this process involves the mitochondrial pathway [92]. Administration of exogenous SM potentiated the effect of antitumor drugs on colon cancer HT-29, HCT-15, and GW-39 cells [93]. It is suggested that SM may be employed for tumor therapy [94, 95]. However, recently it has been reported that SM isolated from vesicles released from the surface of tumor cells promoted malignant growth, metastases, invasiveness, motility of endothelial cells, and angiogenesis [96]. Purified bovine brain SM caused a similar effect [96]. Differences in SM effects in the gut and other cell types may be attributed to different pathways of its metabolism. In the gut, alkaline sphingomyelinase cleaves SM with formation of Cer, responsible for manifestation of the suppressor effect. It is possible that in other tumor cells SM deacylation yields sphingosine-1-phosphocholine (see Scheme), which promotes metastases by increasing cell elasticity [97].

Gangliosides (sialoglycosphingolipids) can also influence malignant tumors. The composition of these biologically active sphingolipids depends on type of tumor cells and stage of their development. Ganglioside content on the surface of almost all tumor cells is higher than in homologous normal cells, and gangliosides normally appearing at various stages of differentiation are often identified in tumor cells (in addition to gangliosides present in normal cells) [98].

Increased formation of such tumor-associated ganglioside as GM3 (sialosyllactosyl ceramide), GD3 (disialosyllactosyl ceramide), GM2, or GD2 was observed in various tumor cells including melanoma, neuroblastoma, lymphoma, and ovary cancer [99]. Antiganglioside vaccines mainly against GM2 ganglioside are employed for tumor therapy (especially melanomas) [100]. *In vitro* studies have also demonstrated that ganglioside antibodies may block proliferation and cause induction of apoptosis of tumor cells. For example, addition of monoclonal antibodies to the cultivation medium resulted in apoptosis of lung cancer small cells containing GD2 and attenuated MAPK activation [101]. Addition of antibodies against GD2 to human lung carcinoma cells caused inhibition of their growth and metastases and also induction of apoptosis in GD2 positive cells [102].

Incubation with monoclonal antibodies against ganglioside GD3 caused significant inhibition of proliferation of all cell lines of human glioma cells [103]. Significant reduction of malignant growth was also noted after addition of GT1b-antibodies to Ehrlich ascites carcinoma cells [104].

Several studies have demonstrated that some gangliosides inhibit the cell cycle and stimulate apoptosis, but they may also promote survival of cells and their growth. These biologically active sphingolipids either transduce signals influencing proliferation or influence functions of receptors and transducers involved in regulation of the cell cycle. The resultant effect is determined by both structure of ganglioside carbohydrate chain and also cell type.

In vitro studies of the effect of various gangliosides on growth of human WM-266-4 metastasizing melanoma revealed that gangliosides GD1b, GT1b, and GQ1b inhibit cell proliferation, decrease protein kinase A activity, and neutralize the effect of interleukin IL-8 [105]. Ganglioside GD1a stimulated proliferation of Swiss 3T3 and normal human fibroblasts in the absence of serum and growth factors [106]. Studies of the inhibitory effect of gangliosides GT1b, GD1a, and GM3 on cell proliferation and phosphorylation of epidermal growth factor receptor in human neuroblastoma NBL-W cell line revealed that all analyzed gangliosides inhibited proliferation in a dose-dependent manner. Ganglioside GD1a was the most potent proliferation suppressor, whereas GT1b was the most effective inhibitor of phosphorylation of epidermal growth factor receptor; GM3 exhibited equal efficacy in inhibition of both receptor phosphorylation and cell proliferation [107].

Monosialoganglioside GM1 is involved in stimulation of cell survival. Treatment of human U-1242 resting glioma cells with ganglioside activated MAPK (mitogen-activated protein kinase) and increased DNA synthesis [108]. Exogenous ganglioside GM1 mimicked or even potentiated many functions of nerve growth factor including maintenance of survival. This ganglioside activates growth factor receptor tyrosine kinase, and this activation inhibits apoptosis [109]. Increased formation of GM1 in rat PC-12 cells increased proliferation [110]. Inhibition of specific sialydase, responsible for shift from more complex gangliosides towards GM1 and conversion of GM3 ganglioside to lactosylceramide, stimulated growth of human neuroblastoma SK-N-MC cells [111]. Treatment of murine neuroblastoma Neuro-2a cells with ganglioside GM1 increased extracellular signal-regulated kinase [112].

Sialosyllactosylceramide exhibits proapoptotic effect with respect to certain cell types especially in the presence of metastases suppressing the protein product of CD-82 gene and its CD-9 analog. Their action was preferentially based on inhibition of cell motility and stimulation of apoptosis induced by simultaneously occurring synthesis and glycosylation of GM3 [113]. In various gut carcinoma cells this ganglioside stimulated apoptosis and increase in endogenous sialydase activity resulted in increased malignancy and tumor metastases by inhibiting Bcl-2 [114]. Artificial increase in GM3 content in human carcinoma HCT-116 cells resulted in a loss of tumor activity of these cells [115]. Treatment of primary cultures of human glioblastoma and rat 9L gliosarcoma cells with ganglioside GM3 sharply reduced the number of cells without any influence on normal brain cells [116]. Various cultures of ependoglioma, glioma, astrocytoma, oligodendroglioma, and ganglioglioma cells were susceptible to treatment with GM3 [116]. In squamous carcinoma cells endogenous accumulation of GM3 inhibited cell proliferation, whereas cleavage of GM3 significantly reduced binding of tyrosine phosphorylated integrin-β1 with epidermal growth factor receptor and promoted cell growth [117]. However, under certain conditions GM3 may promote cell survival: GM3-synthase DNA transfection of cells of a J5 clone of Lewis lung carcinoma resulted in their resistance to apoptosis induced by serum withdrawal [118].

Ganglioside GD3 stimulates proliferation. For example, in rat F-11 cells growth suppression was observed during reduction of GD3-synthase expression and decrease in GD3 concentration [119-121]. Insertion of the GD3-synthase gene to the J5 subclone of 3LL cells (Lewis lung carcinoma) increased proliferation [118]. A similar effect was observed in hamster melanoma cells [122].

Although it is suggested that gangliosides act as extracellular modulators, certain evidence exists that they may act as intracellular messengers as well [123]. Results of recent studies indicate that ganglioside GD3 is a regulator of apoptosis; it is involved in regulation of the mitochondrial pathway of cell death [124, 125]. Immune electron and laser confocal microscopy revealed interaction and accumulation of GD3 in mitochondria of human lymphoblast CEM cells [126] and HT-29 cells [127] subjected to treatment with N-acetylceramide or tumor necrosis factor-α. Studies of mechanisms underlying the effects of this ganglioside revealed that GD3 is involved into Fas-mediated signal transduction. Activation of Fas caused downstream activation of acidic sphingomyelinase. GD3 is rapidly synthesized from the forming pool of Cer. This results in change of membrane potential, permeability of mitochondrial membranes, release of cytochrome *c*, apoptosis inducing factor, and activation of mitochondrial caspases involved in transduction of the apoptotic signal [126-128]. It has recently been found that ganglioside GD3 can increase sensitivity of human hepatoblastoma HepG-2 cells to antitumor therapy. The latter is attributed to the attenuating effect of GD3 on NF-κB mediated survival signals [129]. Ionizing irradiation and daunorubicin activated NF-κB, whereas preincubation of HepG-2 cells with GD3 attenuated NF-κB

mediated induction of genes, and this was accompanied by stimulation of apoptosis [129].

Numerous studies have revealed a correlation between ganglioside compositions of tumors and their metastasizing potential; ganglioside composition also differs in tumor cells and their metastases. Murine melanoma MEB4 cells synthesize GM3 as the major ganglioside. Inhibition of glucosylceramide synthetase was accompanied by reduction in malignancy and metastasizing potential of these cells [130]. Treatment of murine melanoma B16LuF1 cells *in vitro* with gangliosides GM2 and GM3, which were isolated from melanoma cells B16LuF5, B16LuF9, and B16LuF10 exhibiting higher metastases activity, increased metastasizing potential of the former [131, 132]. The metastasizing potential of kidney sarcoma RCC cells also depended on expression of ganglioside GM3 [133]. Inhibition of glucosylceramide synthase decreased endogenous gangliosides in B16 and MEB4 melanomas and their metastasizing potential [134].

Human and mouse urinary bladder tumors also exhibited dependence of invasing properties on ganglioside GM3 content: superficial tumors contained more ganglioside than invasive tumors. Cell clones transfected with GM3 synthase and producing increased amounts GM3 were characterized by reduced proliferation, metastases, and invasiveness. Exogenously added GM3 inhibited invasive potential of human T-24 and KK-47 cell lines. Thus, the authors believe that ganglioside GM3 amount may be an indicator of tumor invasiveness [135-137].

Increased synthesis and shedding of large amounts of gangliosides into the external medium are characteristic features of tumor cells. Shedding is an important factor for infiltration and tumor metastases and also for the inhibition of the immune system. Combined cultivation of kidney tumor cells SK-RC-45 and human leukemic Jurkat cells resulted in apoptosis of the Jurkat cells. Apoptosis was partially or completely prevented by inhibition of ganglioside synthesis in the kidney cells due to changes in signal transduction including reduced expression of Blc-2, induction of cytochrome *c* release from mitochondria, and activation of caspases 9 and 3 [138].

Consequently, some gangliosides (e.g., GD3) are mainly associated with cell death, whereas others (e.g. GM1 and GM3) with cell survival. So the ratio of these gangliosides is very important in these cells.

Summarizing all the abovementioned data on the role of biologically active sphingolipids in tumor process, we can see that these sphingolipids act at intracellular and extracellular levels. Inside cells some sphingolipids act as second messengers and inhibit proliferation and stimulate apoptosis (sphingosine, ceramide, ganglioside GD3). Others stimulate proliferation and inhibit apoptosis (sphingosine-1-phosphate, glucosylceramide). Some sphingolipids (sphingomyelin) can cause suppressor or stimulating effects depending on tumor type. Extracellular sphingolipids (sphingosine-1-phosphate, gangliosides) interact with specific receptors and are actively involved in metastases, invasiveness, and tumor angiogenesis. Sphingolipids also determine resistance of tumor cells to antitumor drugs at least in some tumor types. Since in any cell all sphingolipids are metabolically related, the tumor process depends on their ratio. Impairments of any metabolic component lead to impairments of their dynamic equilibrium, and this determines subsequent fate of the cell.

Thus, results of recent studies indicate that biologically active sphingolipids play an important role in pathogenesis of therapy of tumors. Taking into consideration the diversity of biological effects of sphingolipids, three main directions of sphingolipid use in antitumor therapy have developed: immune therapy by vaccines containing antibodies against gangliosides GM2, GM3, GD2, GD3 (this is already employed now [100, 139, 140]); administration of sphingolipids (Cer, SM) inhibiting proliferation or stimulating apoptosis; inhibition of enzymes promoting formation of sphingolipids blocking apoptosis and inducing proliferation of tumor cells.

It is clear that additional studies in this field will have not only theoretical but also practical importance.

REFERENCES

1. Hannun, Y. A., Loomis, C. R., Merrill, A. H., Jr., and Bell, R. M. (1986) *J. Biol. Chem.*, **261**, 12604-12609.
2. Alesenko, A. V. (1998) *Biochemistry (Moscow)*, **63**, 62-68.
3. Hannun, Y. A., Obeid, L. M., and Dbaibo, G. S. (1996) *Handbook Lipid Res.*, **8**, 177-204.
4. Gomez-Munoz, A. (1998) *Biochim. Biophys. Acta*, **1391**, 92-109.
5. Pyne, S., and Pyne, N. J. (2000) *Biochem. J.*, **349**, 385-402.
6. Hla, T. (2003) *Pharmacol. Res.*, **47**, 401-407.
7. Dyatlovitskaya, E. V., and Kandyba, A. G. (2004) *Bioorg. Khim.*, **30**, 227-233.
8. Desai, N. N., Carlson, R. O., Mattie, M. E., Olivera, A., Buckley, N. E., Seki, T., Brooker, G., and Spiegel, S. (1993) *J. Cell Biol.*, **121**, 1385-1395.
9. Berger, A., Bittman, R., Schmidt, S., and Spiegel, S. (1996) *Mol. Pharmacol.*, **50**, 451-457.
10. Meyer zu Heringdorf, D., Himmel, H. M., and Jakobs, K. H. (2002) *Biochim. Biophys. Acta*, **1582**, 178-189.
11. Hannun, Y. A., and Linardic, C. M. (1993) *Biochim. Biophys. Acta*, **1154**, 223-236.
12. Hannun, Y. A. (1994) *J. Biol. Chem.*, **269**, 3125-3128.
13. Radin, N. S. (2001) *Eur. J. Biochem.*, **268**, 193-204.
14. Radin, N. S. (2003) *Biochem. J.*, **371**, 243-256.
15. Kolesnick, R. (2002) *J. Clin. Invest.*, **110**, 3-8.
16. Perry, D. K., and Kolesnick, R. N. (2003) *Cancer Treat. Res.*, **115**, 345-354.
17. Radin, N. S. (2004) *Eksp. Onkol.*, **26**, 3-10.
18. Reynolds, C. P., Maurer, B. J., and Kolesnick, R. N. (2004) *Cancer Lett.*, **206**, 169-180.
19. Cuvillier, O., and Levade, T. (2003) *Pharm. Res.*, **47**, 439-445.

20. Ogretmen, B., and Hannun, Y. A. (2004) *Nat. Rev. Cancer*, **4**, 604-616.
21. Zhang, H., Bucley, N. E., Gibson, K., and Spiegel, S. (1990) *J. Biol. Chem.*, **265**, 76-81.
22. Ahn, E. H., and Schroeder, J. J. (2002) *Exp. Biol. Med.*, **227**, 345-353.
23. Sweeney, E. A., Sakakura, C., Shirahama, T., Masamune, A., Ohta, H., Hakomori, S., and Igarashi, Y. (1996) *Int. J. Cancer*, **66**, 358-366.
24. Jarvis, W. D., Fornari, F. A., Traylor, R. S., Martin, H. A., Kramer, L. B., Erukulla, R. K., Bittman, R., and Grant, S. (1996) *J. Biol. Chem.*, **271**, 8275-8284.
25. Nava, V. E., Cuvillier, O., Edsall, L. C., Kimura, K., Milstien, S., Gelmann, E. P., and Spiegel, S. (2000) *Cancer Res.*, **60**, 4468-4474.
26. Cuvillier, O., Nava, V. E., Murthy, S. K., Edsall, L. C., Levade, T., Milstien, S., and Spiegel, S. (2001) *Cell Death Differ.*, **8**, 162-171.
27. Cuvillier, O., Edsall, L., and Spiegel, S. (2000) *J. Biol. Chem.*, **275**, 15691-15700.
28. Faucher, M., Girones, N., Hannun, Y. A., Bell, R. M., and Davis, R. J. (1988) *J. Biol. Chem.*, **263**, 5319-5327.
29. Dyatlovitskaya, E. V. (1998) *Bioorg. Khim.*, **24**, 723-730.
30. Andreasyan, G. O., Malykh, Ya. N., and Dyatlovitskaya, E. V. (1996) *Vopr. Med. Khim.*, **42**, 248-253.
31. Riboni, L., Campanella, R., Bassi, R., Villani, R., Gaini, S. M., Martinelli-Boneschi, F., Viani, P., and Tettamanti, G. (2002) *Glia*, **39**, 105-113.
32. Selzner, M., Bielawska, A., Morse, M. A., Rudiger, H. A., Sindram, D., Hannun, Y. A., and Clavien, P. A. (2001) *Cancer Res.*, **61**, 1233-1240.
33. Koyanagi, S., Kuga, M., Soeda, S., Hosoda, Y., Yokomatsu, T., Takechi, H., Akiyama, T., Shibuya, S., and Shimeno, H. (2003) *Int. J. Cancer*, **105**, 1-6.
34. Kandyba, A. G., Koblyakov, V. A., Somova, O. G., and Dyatlovitskaya, E. V. (2004) *Biochemistry (Moscow)*, **69**, 497-500.
35. Sawada, M., Nakashima, S., Kiyono, T., Yamada, J., Hara, S., Nakagawa, M., Shinoda, J., and Sakai, N. (2002) *Exp. Cell Res.*, **273**, 157-168.
36. Colell, A., Coll, O., Mari, M., Fernandez-Checa, J. C., and Garcia-Ruiz, C. (2002) *FEBS Lett.*, **526**, 15-20.
37. Akao, Y., Kusukabe, S., Banno, Y., Kito, M., Nakagawa, Y., Tamiya-Koizumi, K., Hatton, M., Sawada, M., Hirabayasi, Y., Ohishi, N., and Nozawa, Y. (2002) *Biochem. Biophys. Res. Commun.*, **294**, 363-370.
38. Wang, X. Z., Beele, R. J., Pwiti, L., Bielawska, A., and Smyth, M. G. (1999) *Cancer Res.*, **59**, 5842-5848.
39. Von Haefen, C., Wieder, T., Gillissen, B., Starck, L., Graupner, V., Dorken, B., and Daniel, P. T. (2002) *Oncogene*, **21**, 4009-4019.
40. Kim, S. S., Chae, H. S., Bach, J. H., Lee, M. W., Kim, K. Y., Lee, W. B., Yung, Y. M., Bonventre, J. V., and Suh, Y. H. (2002) *Oncogene*, **21**, 2020-2028.
41. Ravid, T., Tsaba, A., Gee, P., Rasooly, R., Medina, E. A., and Goldcom, T. (2003) *Am. J. Physiol. Lung Cell Mol. Physiol.*, **284**, 1082-1092.
42. Fillet, M., Bentires-Alj, M., Deregowski, V., Greimers, R., Gielen, J., Piette, J., Bours, V., and Merville, M. P. (2003) *Biochem. Pharmacol.*, **65**, 1633-1642.
43. Kurinna, S. M., Tsao, C. C., Nica, A. F., Jiffar, T., and Ruvolo, P. P. (2004) *Cancer Res.*, **64**, 7852-7856.
44. Sugiki, H., Hozumi, Y., Maeshima, H., Katagata, Y., Mitsuhashi, Y., and Kondo, S. (2000) *Br. J. Dermatol.*, **143**, 1154-1163.
45. Mimeault, M. (2002) *FEBS Lett.*, **530**, 9-16.
46. Lopez-Marure, R., Gutierrez, G., Mendoza, C., Ventura, J. L., Sanchez, L., Reyes Maldonado, E., Zentella, A., and Montano, L. F. (2002) *Biochem. Biophys. Res. Commun.*, **293**, 1028-1036.
47. Han, W. S., Yoo, J. Y., Youn, S. W., Kim, D. S., Park, K. C., Kim, S. Y., and Kim, K. H. (2002) *J. Dermatol. Sci.*, **30**, 110-119.
48. Kimura, K., Markowski, M., Edsall, L. C., Spiegel, S., and Gelmann, E. P. (2003) *Cell Death Differ.*, **10**, 240-248.
49. Payne, S. G., Milstien, S., and Spiegel, S. (2002) *FEBS Lett.*, **531**, 51-57.
50. Spiegel, S., and Milstien, S. (2003) *Biochem. Soc. Trans.*, **31**, 1216-1219.
51. Hla, T. (2003) *Pharm. Res.*, **47**, 401-407.
52. Spiegel, S., and Milstien, S. (2003) *Nat. Rev. Mol. Cell. Biol.*, **4**, 397-407.
53. Watterson, K., Sankala, H., Milstien, S., and Spiegel, S. (2003) *Progr. Lipid Res.*, **42**, 344-357.
54. Spiegel, S., and Milstien, S. (2002) *J. Biol. Chem.*, **277**, 25851-25854.
55. Cuvillier, O., and Levade, T. (2001) *Blood*, **98**, 2828-2836.
56. Kim, D. S., Hwang, E. S., Lee, J. E., Kim, S. Y., and Park, K. C. (2003) *Cell Signal.*, **15**, 919-926.
57. Xia, P., Gamble, J. H., Wang, L. J., Pitson, S. M., Moretti, P. A., Wattenberg, H. W., Pandrea, R. J., and Vadas, M. A. (2000) *Curr. Biol.*, **10**, 1527-1530.
58. Edsall, L. C., Cuvillier, O., Twitty, S., Spiegel, S., and Milstien, S. (2001) *J. Neurochem.*, **76**, 1573-1584.
59. Nava, V. E., Hobson, J. P., Murthy, S., Milstien, S., and Spiegel, S. (2002) *Exp. Cell Res.*, **281**, 115-127.
60. Maceyka, M., Payne, S. G., Milstien, S., and Spiegel, S. (2002) *Biochim. Biophys. Acta*, **1585**, 193-201.
61. Sadahira, Y., Ruan, F., Hakomori, S., and Igarashi, Y. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 9686-9690.
62. Kawa, S., Kimura, S., Hakomori, S., and Igarashi, Y. (1997) *FEBS Lett.*, **420**, 196-200.
63. Wang, F., van Brocklyn, J. R., Edsall, L., Nava, V. E., and Spiegel, S. (1999) *Cancer Res.*, **59**, 6185-6191.
64. Van Brocklyn, J. R., Young, N., and Roof, R. (2003) *Cancer Lett.*, **199**, 53-60.
65. Spiegel, S., English, D., and Milstien, S. (2002) *Trends Cell Biol.*, **12**, 236-242.
66. Hobson, J. P., Rosenfeldt, H. M., Barak, L. S., Olivera, A., Poulton, S., Caron, M. G., Milstien, S., and Spiegel, S. (2001) *Science*, **291**, 1800-1803.
67. Yamaguchi, H., Kitayama, J., Takuwa, N., Arikawa, K., Inoki, I., Takehara, K., Nagawa, H., and Takuwa, Y. (2003) *Biochem. J.*, **374**, 715-722.
68. Ankawa, K., Takuwa, N., Yamaguchi, H., Sugimota, N., Kitayama, J., Nagawa, H., Takehara, K., and Takuwa, Y. (2003) *J. Biol. Chem.*, **278**, 32841-32851.
69. Wang, F., van Brocklyn, J. R., Hobson, J. P., Movafagh, S., Zukovska-Grojec, Z., Milstien, S., and Spiegel, S. (1999) *J. Biol. Chem.*, **274**, 35343-35350.
70. Panetti, T. S., Nowlen, J., and Mosher, D. F. (2000) *Atherosclerosis, Thrombosis and Vascular Biology*, **20**, 1013-1019.
71. Morii, T., and Weissbach, L. (2003) *Biochem. Biophys. Res. Commun.*, **310**, 884-888.

72. Sietsma, H., Dijkhuis, A. J., Kamps, W., and Kok, J. W. (2002) *Neurochem. Res.*, **27**, 665-674.
73. Ruvolo, P. P. (2003) *Pharm. Res.*, **47**, 383-392.
74. Sietsma, H., Veldman, R. J., and Kok, J. W. (2001) *J. Membr. Biol.*, **181**, 153-162.
75. Liu, Y. Y., Han, T. Y., Giuliano, A. E., and Cabot, M. C. (2001) *FASEB J.*, **15**, 719-730.
76. Morjani, H., Aouali, N., Belhoussine, R., Veldman, R. J., Levade, T., and Mangait, M. (2001) *Int. J. Cancer*, **94**, 157-165.
77. Veldman, R. J., Klappe, K., Hinrichs, J., Hummel, L., van der Schaaf, G., Sietsma, H., and Kok, J. W. (2002) *FASEB J.*, **16**, 1111-1113.
78. Lucci, A., Han, T. Y., Liu, Y. Y., Giuliano, A. E., and Cabot, M. C. (1999) *Int. J. Oncol.*, **15**, 541-546.
79. Ogretmen, B., and Hannun, Y. A. (2001) *Drug Resistance Updates*, **4**, 368-377.
80. Itoh, M., Kitano, T., Watanabe, M., Kondo, T., Yabu, T., Taguchi, Y., Iwai, K., Tashima, M., Uchiyama, T., and Okazaki, T. (2003) *Clin. Cancer Res.*, **9**, 415-423.
81. Olschefski, R. S., and Ladisch, S. (2001) *Int. J. Cancer*, **93**, 131-138.
82. Liu, Y. Y., Han, T. Y., Yu, J. Y., Bitterman, A., Le, A., Giuliano, A. E., and Cabot, M. C. (2004) *J. Lipid Res.*, **45**, 933-940.
83. Weiss, M., Hettmer, S., Smith, P., and Ladisch, S. (2003) *Cancer Res.*, **63**, 3654-3658.
84. Liu, Y. Y., Han, T. Y., Giuliano, A. E., Ichikawa, S., Hirabayashi, Y., and Cabot, M. C. (1999) *Exp. Cell Res.*, **252**, 464-470.
85. Veldman, R. J., Mita, A., Cuvillier, O., Garcia, V., Klappe, K., Medin, J. A., Campbell, J. D., Carpentier, S., Kok, J. W., and Levade, T. (2003) *FASEB J.*, **17**, 1144-1146.
86. Grazide, S., Terrisse, A. D., Lerouge, S., Laurent, G., and Jaffrezou, J. P. (2004) *J. Biol. Chem.*, **279**, 18256-18261.
87. Norris-Cervetto, E., Callaghan, R., Platt, F. M., Dwek, R. A., and Butters, T. D. (2004) *J. Biol. Chem.*, **279**, 40412-40418.
88. Schmelz, E. M., Dillehay, D. L., Webb, S. K., Reiter, A., Adams, J., and Merrill, A. H., Jr. (1996) *Cancer Res.*, **56**, 4936-4931.
89. Berra, B., Colombo, I., Sottocornola, E., and Giacosa, A. (2002) *Eur. J. Cancer Prev.*, **11**, 193-197.
90. Lemonnier, L. A., Dillehay, D. L., Vespremi, M. J., Abrams, J., Brody, E., and Schmelz, E. M. (2003) *Arch. Biochem. Biophys.*, **419**, 129-138.
91. Hertervig, E., Nilsson, A., Cheng, Y., and Duan, R. D. (2003) *J. Cancer Res. Clin. Oncol.*, **129**, 577-582.
92. Birbes, H., El Bawab, S., Hannun, Y. A., and Obeid, L. M. (2001) *FASEB J.*, **15**, 2669-2679.
93. Modrak, D. E., Rodriguez, M. D., Goldenberg, D. M., Lew, W., and Blumenthal, R. D. (2002) *Int. J. Oncol.*, **20**, 379-384.
94. Modrak, D. E., Lew, W., Goldenberg, D. M., and Blumenthal, R. (2000) *Biochem. Biophys. Res. Commun.*, **268**, 603-606.
95. Cutler, R. G., and Mattson, M. P. (2001) *Mechanism Ageing Devel.*, **122**, 895-908.
96. Kim, C. W., Lee, H. M., Lee, T. H., Kang, C., Kleinman, H. K., and Gho, Y. S. (2002) *Cancer Res.*, **62**, 6312-6317.
97. Beil, M., Micoulet, A., von Wichert, G., Paschke, S., Walther, P., Omary, M. B., van Veldhofen, P. P., Gern, U., Wolff-Hieber, E., Eggerman, J., Waltenberger, J., Adler, G., Spatz, J., and Seufferlein, T. (2003) *Nat. Cell Biol.*, **5**, 803-811.
98. Fredman, P., Hedberg, K., and Brezicka, T. (2003) *BioDrugs*, **17**, 155-167.
99. Hanai, N., Nakamura, K., and Shitara, K. (2000) *Cancer Chemother. Pharmacol.*, **46**, Suppl., S13-S17.
100. Wolchok, J. D., and Livingston, P. O. (2001) *Lancet Oncol.*, **2**, 205-211.
101. Yoshida, S., Fukumoto, S., Kawaguchi, H., Sato, S., Ueda, R., and Furukawa, K. (2001) *Cancer Res.*, **61**, 4244-4252.
102. Nakamura, K., Hanibuchi, M., Yano, S., Tanaka, Y., Fujino, I., Inoue, M., Takezawa, T., Shitara, K., Sone, S., and Hanai, N. (1999) *Cancer Res.*, **59**, 5323-5330.
103. Hedberg, K. M., Dellheden, B., Wikstrand, C. J., and Fredman, P. (2000) *Glycoconj. J.*, **17**, 717-726.
104. Saha, S., and Mondal, S. (2000) *Ind. J. Exp. Biol.*, **38**, 1207-1216.
105. Kanda, N., Nakai, K., and Watanabe, S. (2001) *J. Invest. Dermatol.*, **117**, 284-293.
106. Li, R., Manela, J., Kong, Y., and Ladisch, S. (2000) *J. Biol. Chem.*, **275**, 34213-34223.
107. Mirkin, B. L., Clark, S. H., and Zhang, C. (2002) *Cell Prolif.*, **35**, 105-115.
108. Van Brocklyn, J. R., Vandenheede, J. R., Fertel, R., Yates, A. J., and Rampersaud, A. A. (1997) *J. Neurochem.*, **69**, 116-125.
109. Ferrari, G., and Greene, L. A. (1998) *Ann. N. Y. Acad. Sci.*, **845**, 263-273.
110. Nishio, M., Tajima, O., Furukawa, K., Urano, T., and Furukawa, K. (2005) *Int. J. Oncol.*, **26**, 191-199.
111. Von Reitzenstein, C., Kopitz, J., Schuhmann, V., and Cantz, M. (2001) *Eur. J. Biochem.*, **268**, 326-333.
112. Singleton, D. W., Lu, C. L., Colella, R., and Roisen, F. J. (2000) *Int. J. Dev. Neurosci.*, **18**, 797-805.
113. Ono, M., Handa, K., Withers, D. A., and Hakomori, S. (1999) *Cancer Res.*, **59**, 2335-2339.
114. Kakugawa, Y., Wada, T., Yamaguchi, K., Yamanami, H., Ouchi, K., Sato, I., and Miyagi, T. (2002) *Proc. Natl. Acad. Sci. USA*, **99**, 10718-10723.
115. Nojiri, H., Yamana, H., Shirouzu, G., Suzuki, T., and Isono, H. (2002) *Cancer Detect. Prev.*, **26**, 114-120.
116. Noll, E. N., Lin, J., Nakatsuji, Y., Miller, R. H., and Black, P. M. (2001) *Exp. Neurol.*, **168**, 300-309.
117. Wang, X. Q., Sun, P., and Paller, A. S. (2003) *J. Biol. Chem.*, **278**, 48770-48778.
118. Uemura, S., Kabayama, K., Noguchi, M., Igarashi, Y., and Inokuchi, J. (2003) *Glycobiology*, **13**, 207-216.
119. Zeng, G., Gao, L., and Yu, R. K. (2000) *Int. J. Cancer*, **88**, 53-57.
120. Zeng, G., Gao, L., Birkle, S., and Yu, R. K. (2000) *Cancer Res.*, **60**, 6670-6676.
121. Zeng, G., Gao, L., Suetake, K., Joshi, R. M., and Yu, R. K. (2002) *Cancer Lett.*, **178**, 91-98.
122. Birkle, S., Gao, L., Zeng, G., and Yu, R. K. (2000) *J. Neurochem.*, **74**, 547-554.
123. Rippo, M. R., Malisan, F., Ravagnan, L., Tomassini, B., Condo, I., Costantini, P., Susin, S. A., Rufini, A., Todaro, M., Kroemer, G., and Testi, R. (2000) *Eur. Cytokine Netw.*, **11**, 487-488.
124. Fernandez-Checa, J. C. (2003) *Biochem. Biophys. Res. Commun.*, **304**, 471-479.

125. Morales, A., Colell, A., Mari, M., Garcia-Ruiz, C., and Fernandez-Checa, J. C. (2004) *Glycoconjug. J.*, **20**, 579-588.
126. Rippo, M. R., Malisan, F., Ravagnan, L., Tomassini, B., Condo, I., Costantini, P., Susin, S. A., Rufini, A., Todaro, M., Kroemer, G., and Testi, R. (2000) *FASEB J.*, **14**, 2047-2054.
127. Colell, A., Morales, A., Fernandez-Checa, J. C., and Garcia-Ruiz, C. (2002) *FEBS Lett.*, **526**, 135-141.
128. Condo, I., and Testi, R. (2000) *Transpl. Int.*, **13**, Suppl. 1, S3-S6.
129. Paris, R., Morales, A., Coll, O., Sanchez-Reyes, A., Garcia-Ruiz, C., and Fernandez-Checa, J. C. (2002) *J. Biol. Chem.*, **277**, 49870-49876.
130. Deng, W., Li, R., Guerrera, M., Liu, Y., and Ladisch, S. (2002) *Glycobiology*, **12**, 145-152.
131. Saha, S., and Mohanty, K. C. (2003) *Ind. J. Exp. Biol.*, **41**, 1253-1258.
132. Saha, S., and Mohanty, K. C. (2004) *Ind. J. Exp. Biol.*, **42**, 976-980.
133. Saito, S., Nojiri, H., Satoh, M., Ito, A., Ohyama, C., and Orikasa, S. (2000) *Tohoku J. Exp. Med.*, **190**, 271-278.
134. Deng, W., Li, R., and Ladisch, S. (2000) *J. Natl. Cancer Inst.*, **92**, 912-917.
135. Kawamura, S., Ohyama, C., Watanabe, R., Satoh, M., Saito, S., Hoshi, S., Gasa, S., and Orikasa, S. (2001) *Int. J. Cancer*, **94**, 343-347.
136. Satoh, M., Ito, A., Nojiri, H., Handa, K., Numahata, K., Ohyama, C., Saito, S., Hoshi, S., and Hakomori, S. I. (2001) *Int. J. Oncol.*, **19**, 723-731.
137. Watanabe, R., Ohyama, C., Aoki, H., Takahashi, T., Satoh, M., Saito, S., Hoshi, S., Ishii, A., Saito, M., and Arai, Y. (2002) *Cancer Res.*, **62**, 3850-3854.
138. Kudo, D., Rayman, P., Horton, C., Cathcart, M. K., Bukowski, R. M., Thornton, M., Tannenbaum, C., and Finke, J. H. (2003) *Cancer Res.*, **63**, 1676-1683.
139. Helling, F., Shang, A., Calves, M., Zhang, S., Ren, S., Yu, R. K., Oettgen, H. F., and Livingston, P. O. (1994) *Cancer Res.*, **54**, 197-203.
140. Alfonso, M., and Zeuthen, J. (1996) *Acta Oncol.*, **35**, 287-295.