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Review

Anticancer Compounds and Sphingolipid Metabolism in the Colon

RUI-DONG DUAN

Gastroenterology Laboratory, Biomedical Centre, B11, Lund University, S-221 84 Lund, Sweden

Abstract. *Sphingomyelin metabolism generates anticancer signals such as ceramide and sphingosine that may inhibit cell proliferation, and induce differentiation and apoptosis. Changes of sphingomyelin metabolism are found to be associated with tumorigenesis in various tissues and a particular link between sphingomyelin metabolism and colon cancer has been indicated. The effects of several anticancer drugs on sphingomyelin metabolism have been examined recently and there is an increasing interest in discovering new drugs taking sphingomyelin as a target. The present review outlines the sphingomyelin metabolism pathway, introduces the evidence linking sphingomyelin to colon cancer, and summarizes the anticancer drugs and dietary factors that affect the metabolism of sphingomyelin and, thus, the production of the anticancer messengers in the colon.*

Lipid metabolites, formed from the mammalian cell membrane in response to cell stimuli or in the intestinal lumen by hydrolytic enzymes, exert numerous physiological functions. There is currently an increasing interest in the biological effects of lipid messengers such as eicosanoids, glycerolipids, sphingolipids, fatty acids and fatty acid amides. Of the various lipids, sphingolipids may be particularly important as a source of multiple signalling molecules, with impact on cancer development, particularly colon cancer. The formation and action of sphingolipid metabolites have become attractive targets for drug research. This short review first outlines sphingolipid metabolism in the intestinal tract and then focuses on anti colon cancer compounds that affect sphingolipid metabolism.

Correspondence to: Rui-Dong Duan, Gastroenterology Lab., Biomedical Centre, B11, Lund University, S-221 84 Lund, Sweden. Tel: +46 46 222 0709, Fax: +46 46 137277, e-mail: Rui-dong.duan@med.lu.se

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Metabolism and signalling effects of sphingolipids

Sphingolipids are composed of a long chain sphingosine backbone, a fatty acid linked to sphingosine at the amino group, and a head group that attaches to sphingosine by substitution of the terminal hydroxyl group. Due to the difference in the length of sphingosine, the saturation degree of the fatty acids and the structure of the head groups, more than 400 sphingolipids have been identified in humans. Although sphingolipids are present in dietary products, most sphingolipids can be synthesized and metabolized in the body, as outlined in Figure 1. The synthesis of all sphingolipids is initiated by a condensation of serine and palmitoyl-CoA. After a series of reactions including reduction, acylation and desaturation, ceramide is formed. Ceramide is located in the centre of the sphingolipid metabolism network. It can gain a phosphocholine head group from phosphatidylcholine and generate sphingomyelin (SM), which is an abundant sphingolipid in the body. It can also be glycosylated and form glycosphingolipids, which may contain different sugars including glucose, galactose, lactose and oligosaccharides. Both SM and some glycosylceramide can be degraded to ceramide by sphingomyelinase (SMase) and glucosidase, respectively. If not going to the synthesis pathway, ceramide will be hydrolyzed by ceramidase to sphingosine, which can be phosphorylated to sphingosine-1-phosphate. Ceramide can also be phosphorylated or acylated to form ceramide-1-phosphate and 1-O-acylceramide, respectively. The major breakdown products of sphingolipids are phosphoethanolamine and palmitaldehyde, which can be reutilized for synthesis of phosphatidylethnolamine and fatty acid, respectively.

It is well known that the metabolism of sphingolipids generates biologically active signals that affect cell proliferation, differentiation and apoptosis. Of these metabolites derived from sphingolipids, ceramide, sphingosine and sphingosine-1-phosphate have been intensively studied. Generally speaking, ceramide and sphingosine are antiproliferative molecules that inhibit cell growth, stimulate

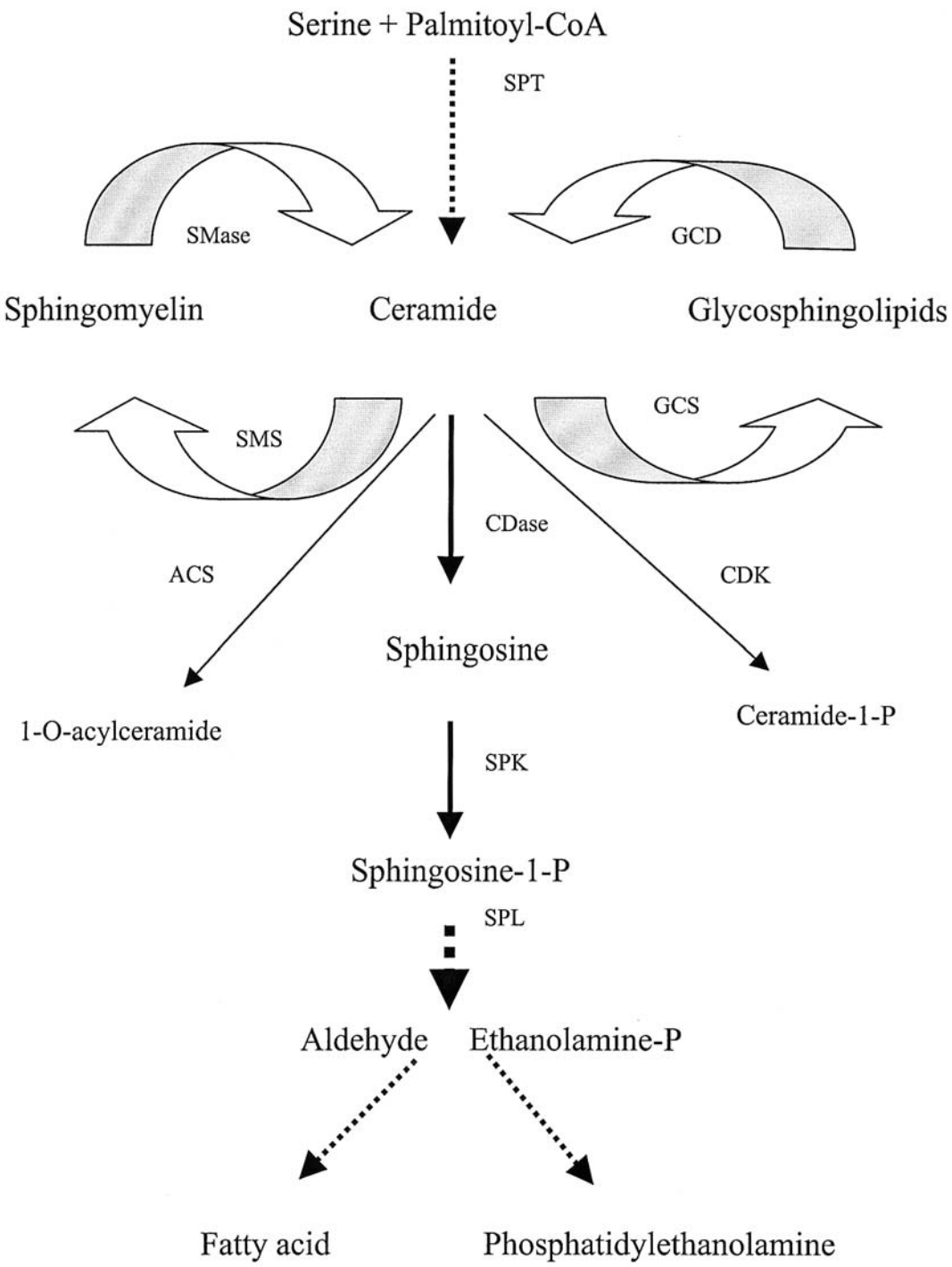

Figure 1. *Outline of the metabolism pathway of sphingolipids. Abbreviations: ACS: acylceramide synthase, CDase: ceramidase, CDK: ceramide kinase, GCD: glucosidase, GCS: glucosylceramide synthase, SMase: sphingomyelinase, SMS: sphingomyelin synthase, SPK: sphingosine kinase, SPL: sphingosine-p lyase, SPT: serine palmitoyltransferase.*

cell differentiation and induce apoptosis, whereas sphingosine-1-phospate promotes cell survival and inhibits apoptosis, and therefore is mitogenic (1) (Figure 2). The mechanisms of the effects of these lipid messengers involve specific protein phosphatases, protein kinases and proteases, which change the expressions or activities of many downstream key molecules and transcriptional factors such as JNK, Akt, MAPK, pRb, c-jun, c-myc, NF-kB and caspases. This review does not cover

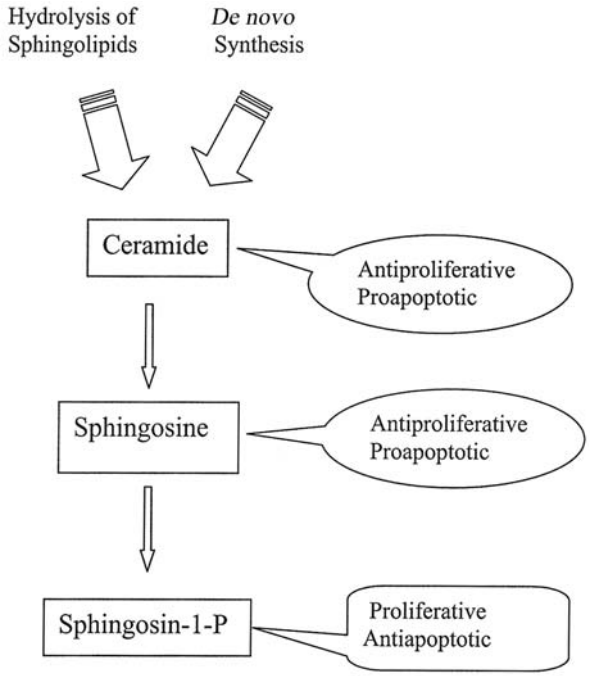

Figure 2. *The biological effects of major sphingolipid metabolites.*

this area and readers are recommended to refer to several excellent review articles published recently (2-5).

Link of sphingolipids to colon cancer

Although the generation of both antiproliferative and proliferative molecules from sphingolipid metabolism indicates a role of sphingolipids in cancer development, a potential link of sphingolipids particularly to colon cancer has emerged. The first indication of such a link is provided by Brasitus *et al.* (6), who showed that accumulation of SM in rat colon preceded tumour development after injection of 1,2-dimethylhydrazine (DMH), a chemical colon carcinogen. Similar results were obtained in human studies (7), where SM was determined in lymph nodes of colon cancer patients, and higher SM levels were found in the positive nodes than in the negative nodes. The increase in SM in the diseased tissues may reflect an inhibited hydrolysis of SM, as the ceramide levels were reduced by 50% in human colon cancer (8).

Dillehey *et al.* first demonstrated that dietary SM inhibited the formation of aberrant crypt foci and increased the ratio of adenoma to carcinomas in mice treated with DMH (9). This anticancer effect was thereafter confirmed and extended in several other studies showing that administration of other types of sphingolipids also inhibits colonic tumorigenesis (10-14). Since sphingolipids are not absorbed intact (15-16), the sphingolipid-induced anticancer effect is probably derived from breakdown products such as ceramide and sphingosine. This is confirmed by both *in vivo* studies, which showed that administration of ceramide mimicked the effect of SM feeding (13), and *in vitro* studies, which showed anticancer effects of ceramide and sphingosine on colon cancer cells (17).

In the intestinal tract, there are enzymes responsible for hydrolysis of sphingolipids (18), of which two are considered to be most important. The first one is alkaline SMase, which is specifically expressed in the intestinal mucosa as an ecto-enzyme (19). The enzyme is able to hydrolyze both SM in the gut lumen and on the cell membrane and is the key enzyme responsible for digestion of dietary SM (16, 20, 21). The enzyme levels are highest in the middle of the small intestine and lower in the colon. Due to the release of the enzyme into the lumen and its protease resistance, a substantial amount of active enzyme passes with the luminal content into the colon. The enzyme may have an inhibitory effect on colonic tumorigenesis. We found that the purified enzyme inhibits cell growth and DNA biosynthesis (22) and that the activity of the enzyme decreases in colon cancer tissues (23-24). The reduction occurs in precancer conditions, as about 20% reduction was demonstrated in long-standing human ulcerative colitis (25), a disease with high risk of colon cancer, and 50% reduction was found in colon sporadic adenomas. At this stage, the activities of acid and neutral SMases were unchanged (23). The reduction of alkaline SMase is further enhanced when adenomas are transformed to carcinoma and the enhancement is associated with reductions of other SMase activities. The mechanism for reduced activity of alkaline SMase in colon cancer is not fully understood. However, an alternative splicing of the enzyme has been demonstrated in HT29 cells, which totally inactivates the enzyme activity (26). Reduction of alkaline SMase may render the mucosa increasingly susceptible to carcinogenic factors. Another enzyme, that is important for sphingolipid hydrolysis in the gut, is intestinal neutral ceramidase, which was recently purified and characterised (27). The intestinal ceramidase distributes along the intestinal tract in parallel with alkaline-SMase (28) and is the major enzyme that catalyzes the breakdown of ceramide in the gut (16, 29). The expression and activity of ceramidase in colon cancer have not been studied in detail. In the intestinal brush border, there is a lactose-phlorizine hydrolase which cleaves glucosylceramide and galactosylceramide to generate ceramide, but has little effect on gangliosides (30-33). In addition, there are several types of glycosylceramidase and glycosidases which are mainly bacterial in origin (33-35). The contribution of these enzymes to the formation of ceramide from cerabrosides and the impact on colonic tumorigenesis are interesting topics for future investigation.

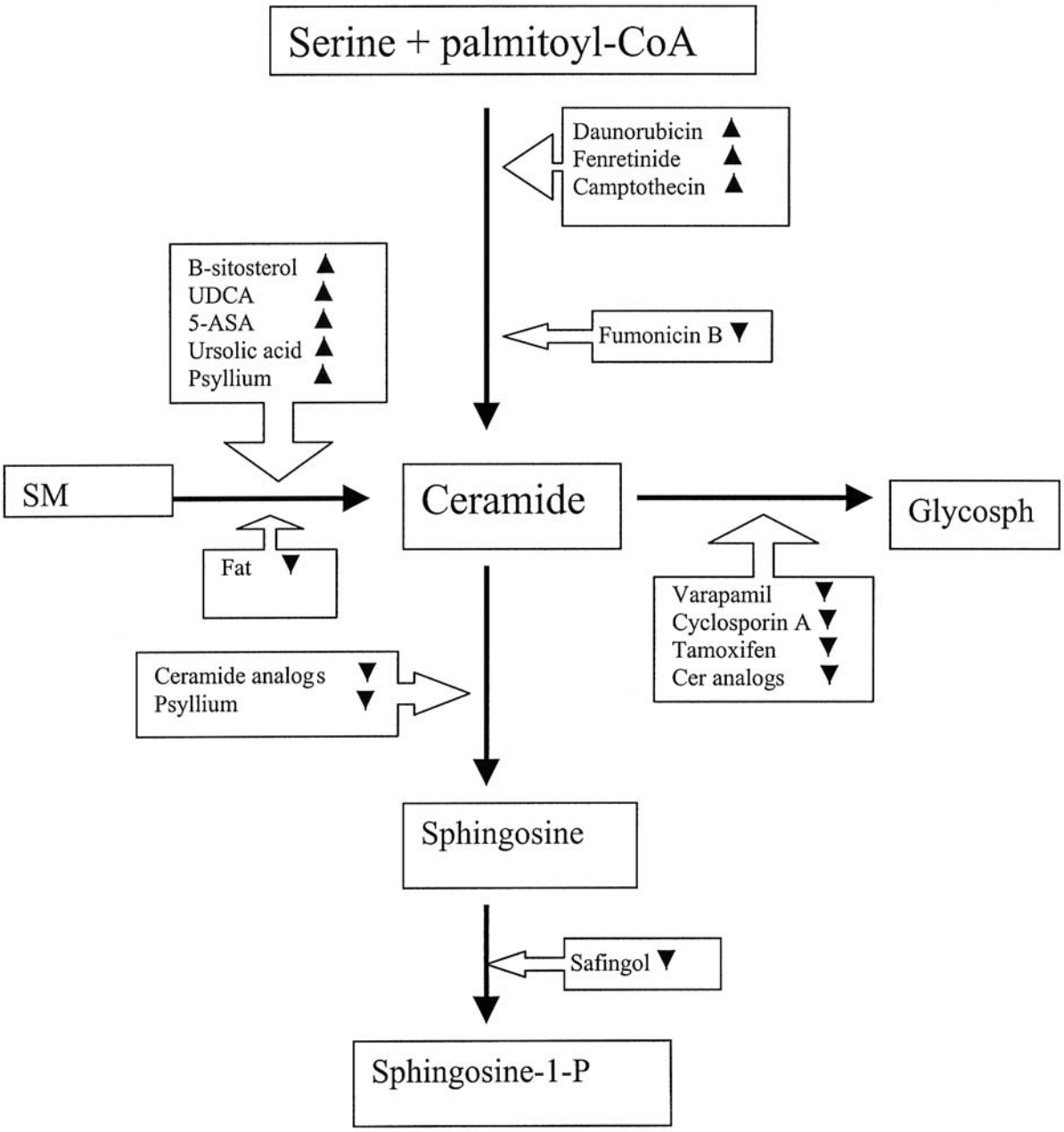

Figure 3. *Compounds that affect the colonic sphingolipid metabolism. ▲ : Stimulation; ▼ :Inhibition.*

Anticancer effects of dietary sphingolipids

The potential link of sphingolipids to colon cancer has stimulated the discovering of novel compounds to prevent or treat colon cancer. Dietary supplementations of sphingolipids including SM, glucosylceramide, lactosylceramide, ceramide, ceramide conjugates and plant sphingolipids have been tested in animal studies for their anticancer effects (9, 13, 14, 36-38). Almost all these compounds were found to be effective in terms of inhibiting the formation of aberrant crypt foci, the migration and proliferation of crypt cells, and the development of adenoma and carcinoma, indicating that the effective component is most probably ceramide or sphingosine, the backbone of all sphingolipids. The dietary sphingolipids were found to be effective in both preventing tumor initiation and progress (37). Age may reduce the sensitivity of colonic cells to supplementary sphingolipids (36). Apart from inhibiting tumor development, dietary SM has been shown to enhance the efficacy of anticancer drugs as well. In nude mice the effects of 5-FU on xenografts of colon cancer HT29 and

HCT15 cell lines was enhanced by administration of SM (39). SM has also been shown to protect colonic cells against the toxic effects of compounds such as deoxycholate (40). For the newborn, SM in the milk stimulates the differentiation and maturation of the intestine (41).

Compounds that stimulate ceramide synthesis in the colonic cells

Ceramide is located at the centre of sphingolipid metabolism. The levels of ceramide in the organs are affected by several pathways: the *de novo* synthesis triggered by serine palmitoyltransferase, the formation of SM and glucosphingolipids by SM synthase and glucosylceramide synthase, the generation from hydrolysis of SM and glucosylceramide by SMase and glucosidase, and the clearance by ceramidase, ceramide kinase and the acylceramide synthase (Figure 1). Scientists have been trying to find agents that affect some of the pathways in the hope of increasing the ceramide levels and inhibiting cancer progress.

The first drug found that affects ceramide metabolism was daunorubicin, which increased ceramide levels in P388 and U937 cells. The effect can be blocked by fumonisin B, the ceramide synthase inhibitor (42), indicating a stimulatory effect of the drug on the biosynthesis of ceramide. Since daunorubicin is not a main anticancer drug against colon cancer, it is unknown whether it also has a similar effect on colon cancer cells. Fenretinide (4-HPR), a synthetic retinoid, has been shown to strongly increase the ceramide levels in neuroblastoma cells and other cells including colon cancer HT29 cells and LoVo cells (43), with less efficacy in colon cancer cells than neuroblastoma cells. However, the effect can be enhanced by combination with safingol, which is an inhibitor of protein kinase C and sphingosine kinase, and which has structural similarity to sphingosine. Camptothecin is a drug on the first line in chemotherapy against colon cancer. Recently, camptothecin and its derivatives were found to increase ceramide levels in HT29 cells and the effects were decreased by the inhibitors of serine palmitoyltransferase and ceramide synthase (44).

Compounds that enhance hydrolysis of sphingolipids in the colon

Ceramide can be generated by hydrolysis of sphingolipids, mainly SM by the enzyme SMase. Non-steroidal anti-inflammatory drugs (NSAID) such as aspirin, sulindac, piroxicam and indomethacin are well known to have anti colon cancer effects with a mechanism related to inhibition of cyclooxygenase (Cox) (45). There are two isoforms of Cox. Cox1 is constitutively expressed, whereas Cox 2 is an inducible enzyme overexpressed in many cancer and inflammatory tissues. Several studies have recently indicated that NSAID may affect hydrolysis of SM by increasing the activity of SMase. Inhibition of Cox will increase the levels of arachidonic acid, which can stimulate neutral SMase activity in HL-60 cells (46) and enhance the activity of purified alkaline SMase from rat intestine (47). 5-Aminosalicylic acid (5-ASA) is a type of anti-inflammatory drug with fewer side-effects as compared with NSAID. 5-ASA has chemopreventive effects on colorectal cancer by a mechanism related to inhibition of NF-κB, MAP kinase, JNK and DNA oxidative damage (48). In animal studies, we found that administration of 5-ASA for 10 days increased alkaline SMase levels selectively in the colon by 78% (49). Several plant compounds with anti-inflammatory properties, such as ursolic acid and boswellic acid, strongly inhibit cell proliferation and induce apoptosis of colon cancer cells *via* a mechanism dependent on caspase 8 activation (50, 51). These compounds mildly increase alkaline SMase in cancer cells (49, 51); the mechanism remains elusive. Since both ursolic and boswellic acids are pentacyclic triterpanoids, these studies may provide useful information towards the discovery of novel drugs that enhance alkaline SMase activity.

Ursodeoxycholic acid (UDCA) is a type of bile salts naturally occurring in human bile in a relatively small amount. Differing from other types of bile acid, UDCA has a cytoprotective effect and has been shown to inhibit colon cancer development in animal studies (52), cell culture studies (53), and in patients with primary sclerosing cholangitis (54). We previously found that, when rats were given UDCA for 10 days, the activity of alkaline SMase in the colonic mucosa increased dose-dependently (55), accompanied by a mild increase in neutral SMase and a reduction of acid SMase activities. The changes of alkaline SMase correlates positively with the activity of caspase 3, the key enzyme that executes apoptosis (56). The changes are a consequence of direct stimulation, since we recently found that UDCA increases the expression of alkaline SMase in Caco-2 cells (unpublished data).

B-Sitosterol is a type of phytosterol widely present in plants and vegetables. Cell culture studies found that β-sitosterol, when incubated with HT29 colon cancer cells, inhibited cell growth and stimulated apoptosis associated with a reduction of SM (57) and an increase in ceramide (58), indicating that the hydrolysis of SM was stimulated. Earlier animal studies showed that β-sitosterol supplementation of the diet inhibited colon cancer development induced by topical exposure of N-methyl-N-nitrosourea (59). Although a large scale cohort study for 6.3 years in the Netherlands did not support a beneficial effect of plant sterol intake (60), the role of β-sitosterol intake *in vivo* in the changes of sphingolipid in the colon of humans is still worthy of investigation.

Other dietary compounds affecting sphingolipid metabolism in the colon.

Whether one can modify SM metabolism and increase the ceramide formation in the colon by intake of dietary compounds is an interesting topic for investigation. An earlier study showed that ingestion of corn oil increased ceramide levels in the rat colon compared with fish oil (61). Arachidonic acid, although it increases the activities of neutral and alkaline SMases in a cell-free system (46), had no detectable effect on SMase activity in the colon when supplemented in the diet (62). Red meat is considered a noxious factor for colon cancer development. The cancer-promoting effect of red meats is probably not related to the influence on SM hydrolysis, as feeding red meat did not change any SMase activity (62). However, whether these dietary compounds affect the *de novo* ceramide synthesis is not known.

We recently demonstrated that the expressions of alkaline SMase and ceramidase are significantly affected by fiber and fat in the diet. Psyllium is a type of water soluble fiber derived from *Plantcago ovata*, and is partly broken down in the colon by bacterial flora. Feeding mice with a semi-synthetic diet containing 10% psyllium for 4 weeks significantly increased alkaline SMase activity and decreased neutral ceramidase activity in the colon (63). The increase in alkaline SMase activity is associated with an increased expression of the enzyme protein. The reciprocal changes of alkaline SMase and neutral ceramidase could elevate the levels of ceramide in the cells and promote apoptosis. In agreement with this speculation, both caspase 3 activity and enzyme protein were significantly increased by psyllium feeding and the changes were in positive correlation with that of alkaline SMase. Differing from psyllium, the water insoluble fiber cellulose did not show a significant effect on SMase or caspase 3 activity in the colon (64). In contrast to psyllium, a high fat diet (53% energy) significantly decreased the expression of alkaline SMase activity by 64% without a significant effect on that of neutral ceramidase (63). The effects of fat can not be reversed by administration of cellulose (65), though partly by that of psyllium (63).

Poly-drug strategy

Ceramide is in the centre of the sphingolipid metabolism network and can be readily metabolised. Apart from the generation of sphingosine, that still has anticancer properties, the metabolism of ceramide by other pathways such as formations of SM, glycosylceramide, ceramide-1-phosphate and 1-0-acylceramide will decrease the levels of ceramide and thus attenuate its anticancer effect. Of particular importance is that cancer cells often have high ability to form glucosylceramide (66, 67) and the over-glycosylation of ceramide is associated with multiple drug resistance in many cells including colon cancer cells (68). An up-regulation of multidrug resistance protein 1 has been recently found to be caused by glucosylceramide (69). A poly-drug strategy, therefore, must be considered in fighting cancer by taking ceramide metabolism as a target. Glucosylceramide synthase can be inhibited by several calcium blockers such as verapamil and tamoxifen and by cyclosporin A (67, 70). Several ceramide analogs have been synthesized and shown to inhibit either ceramide glycosylation or ceramidase activity (8, 71) and used in colon cancer studies. For detailed discussion of these aspects, readers are recommended to read the excellent reviews recently written by Radin (72, 73).

The identification of compounds that inhibit colon cancer based on sphingolipid metabolism is just beginning. Figure 3 summarizes the major compounds and their functions discussed in this review. We foresee rapid progress in this field when the metabolism and function of different sphingolipids have been better understood, and the detailed signalling transduction pathways related to metabolites of sphingolipid have been established.

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