Chapter

Understanding Sphingolipids Metabolism in Colorectal Cancer

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Abstract

Colorectal cancer is the fourth most frequently diagnosed cancer and one of the leading causes of cancer death around the world. Patients with locally advanced rectal cancer are treated with a combination of radiotherapy, chemotherapy, and surgery. Treatment response can be quite variable—some with complete response, while others show little or no response—and pathologic response has become a significant predictor of good oncologic outcome. The knowledge of the molecular pathways in colorectal cancer is increasing. However, unfortunately, it still fails to find some more precise method to select and tailor patients to different treatment approaches and overcome treatment resistance. Recent investigations showed that sphingolipids play an essential role in cancer biology and can influence treatment response and aggressiveness. It is of utmost importance to understand sphingolipids' metabolism in colorectal cancer and how it affects tumor biology and response to treatment.

Keywords: locally advanced rectal cancer, neoadjuvant treatment, response to treatment, biomarkers, sphingolipids metabolism

1. Introduction

Colorectal cancer is the fourth most frequently diagnosed cancer and one of the leading causes of cancer death around the World [1]. Unfortunately, despite significant advances in treatment, there has still not been a proportional improvement in survival [2, 3]. This aspect is related to diagnosing and treating neoplasms at a more advanced stage. Although considered a single entity, locally advanced colorectal cancer should be differently treated if located in the colon or mid/lower rectum [4].

In the case of locally advanced rectal cancer (LARC), in part due to its anatomical location, multimodal therapy, and neoadjuvant therapy, in particular, plays a leading role. The optimal treatment plan for patients with rectal cancer can be a complex and highly individualized process. It usually results in multimodal therapy that combines radiation therapy, chemotherapy, and surgery [5]. Although early stages can be treated with surgery alone, more advanced stages (stages II and III) typically are treated with neoadjuvant chemoradiotherapy (CRT) before surgery to decrease the risk of recurrence and optimize oncologic outcomes. The Swedish Rectal Cancer Trial, the Dutch Colorectal Cancer Group trial, and The German Rectal Cancer Group all showed that on long-term follow-up, neoadjuvant CRT was found to improve 5-year local recurrence rates, been the overall survival effect not so evident [6–8]. Response

to neoadjuvant CRT can be quite variable; some have minimal response while others have a complete clinical response [9]. Pathologic response has since become an established surrogate marker of long-term survival and a useful oncologic benchmark [10, 11]. About 20% of LARC patients have a pathologic complete response. In comparison, therapeutic resistance is evident in 80% of the cases and contributes to surgical failure, disease recurrence, and poor prognosis [12]. This discrepancy is of utmost importance because one cannot forget that the associated morbidity of these strategies cannot be underestimated.

Despite increasing knowledge of the molecular signaling pathways implicated in rectal cancer, therapeutic outcomes are still only moderately successful in comparison. To change the therapeutic paradigm, LARC patients must be integrated into clinical algorithms tailoring therapy for individual patients by either identifying more effective strategies or by omitting ineffective treatments to avoid unnecessary toxicity [12, 13].

As one should note, the high rate of resistance demonstrated by the low complete response in most rectal cancer patients must lead the scientific community to explore novel molecular strategies to enhance conventional therapy.

Recent investigations showed that bioactive sphingolipids play a significant role in the colon and rectal cancer tumorigenesis, signaling mechanisms, and response to treatment as they can influence the impact and effectiveness of radio and chemotherapy. Understanding the molecular patterns and the relation between sphingolipids and CRT should provide valuable information regarding tumor survival mechanisms and, this way, pursue novel therapeutic targets.

2. Sphingolipids' metabolism and cancer

Sphingolipids are structural molecules of cell membranes with an essential role in barrier and fluidity functions [14]. They have been implicated in many physiologic and pathologic processes, such as cell growth, cell death, cell adhesion, proliferation, stress, inflammatory responses, differentiation, migration, invasion, and/or metastasis, by controlling signaling functions within the signal transduction network of cancer cell [13, 15–19]. The two central bioactive lipids, ceramide and sphingosine-1-phosphate (S1P), have opposing roles in regulating cancer cell death and survival [19]. Ceramide has been shown to mediate cell cycle arrest and cell death in response to cell stress [14, 20]. S1P has been shown to promote cell survival and proliferation [14, 18, 20, 21].

During the past decades, information regarding almost all major enzymes involved in sphingolipid metabolism was gathered, which has provided data that shows that these metabolic enzymes highly regulate the abundance of sphingolipids and their role in different biologic pathways [22]. Additional complexity derives from multiple isoforms of those enzymes that can vary in subcellular location and pH requirements, which results in different metabolic products. For instance, different ceramide synthases can produce ceramides with different fatty acid chains, which will have distinct biologic roles [12]. One should also find that different isoforms of sphingosine kinase, which generates S1P, have different localizations and functions.

Cellular stress induced by chemotherapy and/or radiation is known to cause pro-cell death mechanisms and tumor suppression, at least partly through the induction of ceramide generation [19]. On the contrary, S1P generation results in resistance to CRT. Given the importance of CRT in the treatment of LARC, understanding the

relation between sphingolipids metabolism and CRT could be of utmost importance in finding new ways to treat these patients more effectively. One must also find that understanding more about the sphingolipids' metabolism may open opportunities to define potential predictive biomarkers for CRT resistance, such as S1P and glucosylceramide, as shown in previous studies with different types of tumors [23, 24].

Cellular stress induces sphingosine and/or ceramide generation by activating the de novo synthesis pathways, sphingomyelin hydrolysis, or the salvage pathway to mediate cancer cell death (**Figure 1**) [14, 25]. By contrast, many tumors exhibit increased ceramide metabolism mainly by increased activities of glycosylceramide synthase (GCS), sphingomyelin synthase (SMS), ceramide kinase (CERK), acid ceramidase (AC), and/or sphingosine kinase (SPHK), which increases the generation of sphingolipids with pro-survival functions [26, 27].

Ceramide consists of a long-chain sphingosine base and an amide-linked fatty acyl chain that varies from 14 to 26 carbons (C) in length [14, 25]. Endogenous ceramides are synthesized via the de novo pathway with the help of ceramide synthases (CERS1-6) [28], which are specialized for ceramide synthesis with different fatty acyl chain lengths. CerS or longevity assurance genes (LASS) [29, 30], a family of six members in mammals with differing tissue expression, are primarily confined to the endoplasmic reticulum (ER). Each CerS1-6 isoform has a unique tissue expression profile and predilection for a fatty acyl CoA with a specific FA chain length. Thus, depending on the CerS family member, distinct sets of ceramides with varying

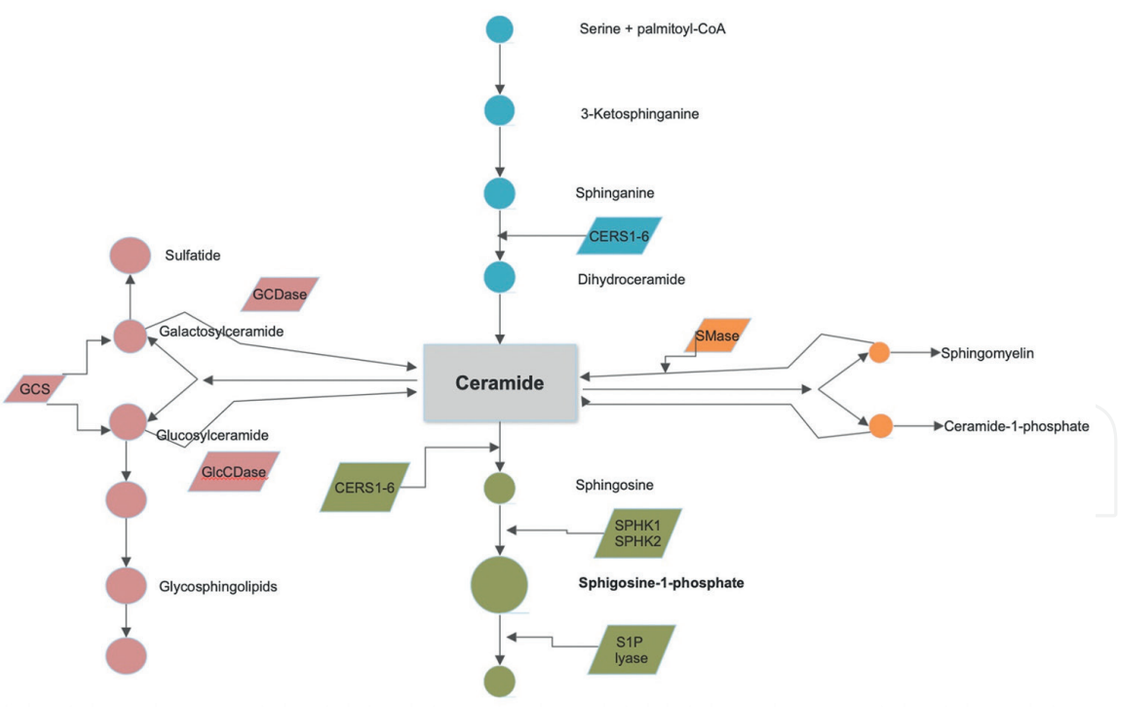

Figure 1.
Sphingolipid metabolism and some of the critical enzymes. De novo synthesis (blue) depends on CERS1-6 activity and it is the central hub of the sphingolipid pathway. Ceramide is also produced by the sphingomyelin hydrolysis (orange), which is dependent on SMase activity. The salvage pathway also relies on CERS1-6 activity (green) that can metabolize free sphingosine to ceramide. Ceramide can be converted to sulfatides by the action of galactosylceramide synthase (GCS). The complex glycosphingolipids are hydrolyzed to glucosylceramide and galactosylceramide. These lipids are then hydrolyzed by beta-glucosidases and beta-galactosidases (GCDase) to regenerate ceramide. CDase activity will metabolize ceramide to sphingosine that, in turn, will lead to S1P unbalancing the scale to a less apoptotic and pro-surviving state. S1P can be broken down by S1P lyase activity exiting the sphingolipid metabolic pathway.

chain lengths are produced [30]. With few exceptions, naturally occurring mammalian ceramides generally possess acyl chain lengths varying between C16 to C24 [31] and its biological activity has only recently become apparent.

Some studies with the administration of exogenous C16-Cer in human colon cancer cell lines showed that it resulted in programmed cell death, suggesting that an increase in endogenous production of C16-Cer could lead to the same effects [32].

Despite these results, one should note that the same ceramide analogs have entirely different effects regarding the type of histological tissue. In the head and neck squamous cell carcinoma cell line, C16:0-Cer had antiapoptotic properties [33], whereas, in HeLa cells, C16:0-ceramide worked as a proapoptotic factor [34]. Ceramides chain length is another critical factor as specific chain lengths can have different effects in different cells. Long-chain and very-long-chain ceramides have shown the opposite effect on the human colon cancer cell line [35].

Moreover, the deficiency of some ceramides may be compensated for by increased expression of others, resulting in an altered synthesis of different ceramide analogs [36].

Ceramide is also generated by sphingomyelinases (SMases, acid, neutral, or alkaline), which mediate sphingomyelin hydrolysis—by far the most abundant sphingolipid in animal cell membranes [31]—or by glucosylceramidase (GlcCDase) and galactosylceramidase (GCDase), which, respectively, catalyze glucosylceramide and/or galactosylceramide breakdown to ceramide [14, 25, 37]. In the salvage pathway, CerSs are responsible for regenerating ceramide from free sphingosine by re-acylation [38].

Ceramide is hydrolyzed by ceramidases (CDases) to yield sphingosine, which is phosphorylated by sphingosine kinases (SPHK1 and SPHK2) to generate S1P [19]. A balance between the proapoptotic properties of ceramide and the antiapoptotic properties of S1P has been termed the ceramide/S1P rheostat and is considered important in balancing cell death and survival in numerous stress situations [39]. S1P engages with five specific G protein-coupled receptors, S1PR1-5, in an autocrine or paracrine manner to elicit pro-survival signaling in various cancer cells [19, 40].

The clinical relevance of sphingolipid metabolism has been established, and it is well known, as demonstrated in the biopathological mechanisms of lysosomal storage diseases (Farber disease, Gaucher disease, Krabbe disease, and Niemann-Pick A, B disease), owing to aberrant accumulation of sphingolipids [19]. Although some of the effects of SLs appear to be cell-specific, generally, increased intracellular levels of ceramides, sphingosines, and also dihydroceramides are mostly connected with the induction of cell cycle arrest and/or cell death. In contrast, the elevated levels of S1P, ceramide-1-phosphate, glucosylceramides, and lactosylceramides seem to be associated with increased cell survival, proliferation, cell adhesion, and promotion of cell migration and/or invasion, events that are related to cancer progression [22]. Until now, the changes in S1P/Cer ratio remain the best-characterized outcome of the alterations of SL metabolism in cancer.

2.1 Biology of cancer and sphingolipid enzymes

Ceramides are essential components of cell membranes, and their presence depends on the equilibrium between production and degradation rates. Different stress stimuli, physiological or pathological, will change the way they act, usually leading to cancer cell death through various mechanisms [36] such as apoptosis, autophagy, and ER stress. In fact, as can be seen by numerous laboratory studies, the

accumulation of sphingolipids represents the great majority of cell changes during apoptosis [36].

In 1993, the induction of apoptosis by ceramide was first demonstrated in leukemic cells by treatment with exogenous ceramide [41]. There are two primary pathways, an intrinsic one (mitochondrial) and an extrinsic one. While the extrinsic one results from the activation of death receptors on the cell surface, the intrinsic pathway is activated by stress stimuli like hypoxia, nutrient deprivation, or DNA damage. Cancer cells can overcome those mechanisms, escape apoptosis, and engage in pro-survival pathways [36, 42].

Despite the proapoptotic action of ceramides in cancer cells, it can also have an opposite behavior in regard to subcellular localization, the type of stress stimuli, and changes in ceramide targets [19].

The abundance of sphingolipid molecules is highly regulated by metabolic enzymes, the altered expression or activity of which has crucial roles in the induction of cancer cell death or survival [19]. 2002 was marked as the year of the discovery of the first mammalian ceramide synthase. Since then, various experiments have indicated that changing the composition of ceramide species alters cell physiology and influences pathology [43].

The discovery and cloning of CERS1-6 were key to understanding the roles of ceramides with different fatty acyl chain lengths in cancer cell signaling. CerS1 and CerS4 preferentially generate ceramide with 18–20-carbon fatty acids (C18–20-Cer), while CerS5 or CerS6 primarily generate ceramide with 14–16-carbon fatty acids (C14-16-Cer), and CerS2 selectively generates ceramides with 22–24-carbon fatty acids. CerS3 is responsible for synthesis of very-long-chain C28-32 ceramides [12].

Phenotypes observed in CerS-deficient mice suggest that ceramides with different fatty acid chain lengths have distinct biologic roles. For example, CerS1 expression was found to be repressed in head and neck cancer cells [44]; In the liver, CerS2-deficiency resulted in a compensatory generation of C16-Cer, which leads to the development of hepatocellular cancer owing to possible defects in apoptosis [45]. C16 ceramide was shown to increase apoptosis in colon cancer cells [46]. Targeting specific CerS can, in theory, shift ceramide composition in cancer cell lines resulting in different cellular responses and signaling pathways. The tissue distribution of CerS varies and likely reflects the need for specific ceramide species for proper signaling and sphingolipid homeostasis in any given tissue [29, 47].

Ceramide is also generated by the hydrolysis of sphingomyelin by SMases – acid, neutral, and alkaline – based on their pH-dependent optimal activity. Data from different studies support the hypothesis that the hydrolysis of sphingomyelin by SMases generates ceramide, which mediates cancer cell death, growth arrest, and/or tumor suppression [19]. In comparison to surrounding normal tissue, SMase activity in colorectal cancer is reduced by 75%, 50%, and 30% for alkSMase, nSMase, and aSMase, respectively [48].

There are three classes of CDases—acid, neutral, and alkaline—responsible for converting ceramide to sphingosine, which was found to be upregulated in various cancer types. Studies with prostate cancer mouse models showed tumor relapse due to radiation resistance induced by ACDase expression [49]. Neutral ceramidase (NCDase) sphingosine release is utilized for S1P biosynthesis by SPHK1 and/or SPHK2, resulting in the inhibition of cell death through reduced levels of proapoptotic ceramide. Colon cancer cells' works demonstrated that NCDase inhibition resulted in autophagy and apoptosis due to ceramide accumulation. In fact, null mice were protected from the development of colon cancer [50].

The two isoforms of sphingosine kinase, SPHK1, and SPHK2, both utilize sphingosine and generate S1P but have significant differences in subcellular localization and function [51]. SPKH1 releases S1P extracellularly, which regulates several cellular processes in an autocrine or paracrine manner, leading to pro-survival mechanisms. SPHK2 appears to have both pro and antiapoptotic functions in regard to the cell type, subcellular localization, and stimuli [51]. Increased expression of SPHK1 mRNA was indicative of poor prognosis and decreased survival in patients with various cancers [52].

SPL function represents a final path and an exit route from the sphingolipid metabolism with the hydrolysis of S1P. In fact, some studies show S1P accumulation in colon cancer tissues due to SPL downregulation [53]. On the contrary, SPL overexpression leads to increased apoptosis through reduced S1P signaling in colon cancer cells [54].

There is ample evidence suggesting that SPHK/S1P signaling pathways are associated with cancer development and metastasis (**Table 1**) [55]. Overexpression of SPHK/S1P signaling is often associated with cancer drug resistance to chemotherapy, radiation therapy, or hormonal therapies in various types of cancers [26]. It is important to note that along with SPHK1, SPHK2 is overexpressed in many human cancers, and based on its cellular localization, it can function as a pro- or antiapoptotic signaling molecule. It was suggested that knockdown of SPHK2 with siRNA or inhibition of SPHK2 activity with the selective pharmacological drugs reduces cancer cell growth, migration, and invasion [56–58] and induces apoptosis by accumulating proapoptotic ceramides. In sharp contrast, it has been recently demonstrated that mitochondrial SPHK2 is proapoptotic [55]. However, more studies need to be performed with specific SPHK2 inhibitors or mitochondrial-targeted SPHK2 that would be beneficial to identify clinically relevant functions of SPHK2.

2.2 Sphingolipids and cancer therapy

The knowledge acquired in recent years regarding sphingolipids metabolism made clear that there are quite a substantial number of different opportunities for cancer cells to escape cell death. In fact, sphingolipid metabolic pathways represent an essential branch of human and pharmacological research in pursuit of novel

Lipids	Mechanism	Functions
S1P	Intracellular Extracellular	Tumor progression
		Metastasis
		Cancer cell survival
		Cell migration
		Angiogenesis
		Inflammation
		Chemokine signaling
		Immune cell trafficking
		Epigenetic regulation

Table 1.
Significant effects mediated by S1P.

therapeutic drugs for cancer patients. About two decades ago, researchers first showed that standard-of-care treatments, for example, chemotherapeutics and radiation, modulate sphingolipid metabolism to increase endogenous ceramides, which kill cancer cells. Strikingly, resistance to these treatments has also been linked to altered sphingolipid metabolism, favoring lipid species that ultimately lead to cell survival [59]. The significant number of chemotherapeutic agents available in clinical practice is, in fact, characterized by the accumulation of sphingolipids in cells [60]. The response to stress induced by chemotherapeutic agents leads to ceramide accumulation, both by sphingomyelin hydrolysis as well as through de novo synthesis of ceramide [61], as described for daunorubicin, etoposide, and gemcitabine [60]. So, inhibiting de novo pathway enzymes leads to decreased ceramide levels, reducing the cytotoxicity of the chemotherapeutics and finally their overall efficacy. In the phase II clinical trial, elevated serum levels of C18 ceramide were markedly associated with improved response to gemcitabine plus doxorubicin combination therapy in patients with recurrent head and neck cancers [62].

Interestingly, altered ceramide levels are not the only biological connection between sphingolipids and chemotherapy; glucosylceramides are increased in breast cancer and in patients who were resistant to chemotherapy. The enzyme that generates glucosylceramide is upregulated in several different tumor types such as lung cancer, breast cancer, and colorectal cancer [63].

Ceramide levels can also be diminished by the action of CDase enzymes which converts ceramide to sphingosine, which, in turn, can be transformed to S1P. *In vitro* and *in vivo* studies have shown that by overexpressing ACDase, tumors are more aggressive and resistant to chemotherapies [64].

In essence, when too much ceramide accumulates and the metaphorical balance overflows, the cell dies (**Figure 2**).

In regard to radiotherapy, one of the first discoveries of the role of ceramide in cell death in radiation subjects was the rapid hydrolysis of sphingomyelin to ceramide by SMase [65]. Notably, ceramide was shown to be the major mediator of cellular stress after radiation exposure [66]. Besides sphingomyelin hydrolysis, raised ceramide levels can also be achieved by induction of de novo synthesis in response to radiation, as seen in Scarlatti F. *et al. in vitro* study with radiation-resistant DU145 prostate cancer cells. Those cells were treated with resveratrol resulting in resensitization to radiation by stimulating the de novo pathway, a finding that was validated when sphingolipid synthesis inhibitors blocked sensitization and reverted DU145 cells to radiation-resistant status [67].

Lastly, ceramide cell levels in response to radiation are also increased by ceramide synthase activity [68]. The current knowledge is that ceramide levels are firstly increased by sphingomyelin hydrolysis and then by CerS activity, 8 to 24 h after radiation therapy [69]. These data suggest that ceramide generation in cancer cells in response to chemotherapy and radiotherapy has an important role in tumor suppression.

Bacterial resistance to antibiotic drugs was first described after the discovery that penicillin prompted bacteria to develop defense mechanisms culminating in the expression of an array of efflux transporters in the outer cell wall [70]. The broad range of substrates used by these transport proteins resulted in coining the term multidrug resistance (MDR) as pathogens can limit the accumulation of diverse drugs targeted against them [31, 71]. Some cancer types harbor intrinsic MDR, most probably due to exogenous expression of drug efflux transport proteins in the tissue of origin. Other cancer types acquire MDR through prolonged

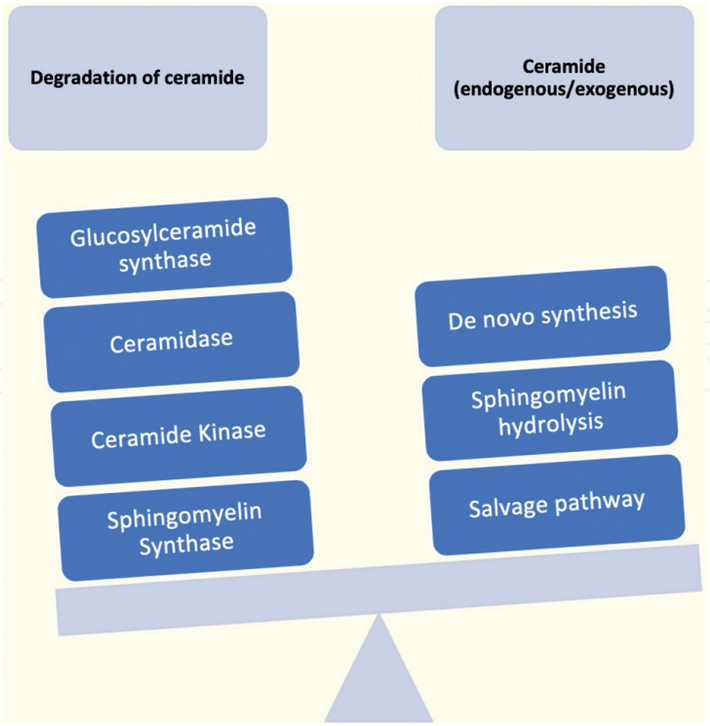

Figure 2.
The accumulation of ceramide (endogenous and exogenous) and degradation of ceramide.

or repeated treatment with chemotherapeutic drugs [72]. An altered glycosphingolipid profile in cancerous versus non-cancerous cells was observed in cell lines transformed by chemicals or viruses and impacted cell growth, intercellular recognition, and cell adhesivity. The conversion of ceramide to glucosylceramide by GCS has been shown to mediate drug resistance in various cancers [23]. Importantly, drug sensitivity was restored when GCS was inhibited or downregulated [73], but not all studies exhibit the dependence of drug resistance on CGS/CluCer [74]. SPHK1 overexpression was reported at intrinsic or acquired resistance to cetuximab in CRC cell lines, xenograft mouse models, and tumors obtained from patients [24] and S1PR1 inhibition using FTY720 sensitized resistant CRC cells and tumors to cetuximab [24]. Hence, while CGS and SPHK1/2 are potential therapeutic targets to overcome drug resistance, increased accumulation of their sphingolipid products—glucosylceramide and S1P, respectively—might be potential predictive biomarkers for chemotherapy resistance in various cancers [19]. Descriptive lipidomic studies may help to identify potential lipid markers of distinct rectal cancer stages.

3. Sphingolipids and colorectal cancer

3.1 Sphingolipids' levels in plasma and tumor tissue

The last decade was fruitful in the investigation of the metabolic switch during tumorigenesis [75]. Lipids are central in different cellular levels of physiology that go from plasmatic and membrane organization, plasticity, and signaling mechanisms [76–78].

Data from the literature indicate that the equilibrium between ceramides of various chain lengths is crucial for cell fate [35]. As noted before, the S1P/Cer ratio changes remain the best-characterized outcome of the alterations of SL metabolism in cancer.

The amount of new information and knowledge regarding sphingolipids in colorectal cancer can hardly be systematized. The best option is to follow the sphingolipids' metabolic pathways and see which alterations are present in cancer cells.

Ceramides and their proportion are different in plasma of patients with CRC and tumor tissue compared with plasma and tissue control levels. On the other hand, plasma ceramide concentration is not directly related to ceramide concentration in tumor tissue. One must also be aware that different chain lengths can have different actions regarding cell localization and the microenvironment. Chen et al. demonstrated increased levels of C16:0 and C24:0 ceramides and reduced levels of both C18 and C20 ceramides in colorectal tumor tissues [79–81]. Levels of C22:0 ceramide were unchanged [80]. Those results were in line with the protein expression and enzymatic activity of SCD1 (Stearoyl-CoA desaturase-1), a key conversion enzyme that regulates lipogenesis. SCD1 inhibition impairs the proliferation of cancer cells probably by cellular endogenous ceramide signals mediation [80]. Another study showed an increased amount of S1P and C14:0 compared to normal tissue and a significantly lower amount of C18:0 and C20:0, as previously noted [36].

The plasma profile of sphingolipids appears to be different than in tissues with the highest concentration in the plasma for C24:0-ceramide and C24:1-ceramide [36]. The concentration for C22:0, C16:0-ceramides, and S1P is smaller but significant [36]. Another study, however, showed significantly higher concentration levels of C16, C18, C18:1, and C24:1-ceramide than those of controls and lower levels of C24-sphingomyelin; there was a relation between these results and stage IV CRC. These results are limited by the small sample size and retrospective design of the study [82]. Markowski et al. divided the patients into two groups regarding their stage and showed that a higher tumor content of C20:0 and C24:0-ceramide was present in the TNM III + IV group. In plasma, there was a statistically significant relation between CRC patients in TNM stage III + IV and higher levels of C16:0 and C18:1-ceramides. Their data raise the possibility that it could be possible to distinguish patients between early and advanced stages based on this model [36]. Taken together, one must note that plasma ceramide concentration is not directly related to ceramide concentration in tumor tissue.

In another study with patients with pulmonary and hepatic metastasis submitted to radiotherapy, it was observed that although pre-treatment levels of ceramides did not correlate with response to treatment, patients with complete response had higher post-treatment total plasma ceramide levels than non-responders [83].

Lymph node invasion was shown to have a positive correlation with C24 ceramide levels in CRC tumor tissues [79]. It was also demonstrated that Sphingosine 1-phosphate (S1P) signaling pathways were associated with lymphangiogenesis [84].

3.2 Sphingolipids enzymes in colorectal cancer

3.2.1 Pro-ceramide metabolic pathways

As mentioned before, sphingolipids' metabolism is regulated through a complex equilibrium between different enzymes' actions, which will, in the end, change the balance between ceramide and S1P. For example, different enzymes will provide

different ceramides, with different actions depending on the tissue and subcellular localization.

The discovery and cloning of CERS1–6 were crucial for understanding the roles of ceramides with different fatty acyl chain lengths in cancer cell signaling. Hartmann et al. showed that overexpression of CerS4 and CerS6 in HCT-116 human colon cancer cells inhibits cell proliferation by upregulation of long-chain ceramides C16:0, C18:0, and C20:0. In contrast, upregulation of CerS2 and concomitant increase of C24:0 and C24:1 promotes cell proliferation [35].

Jang et al. revealed that all four CerS genes were significantly upregulated in CRC tissues compared with corresponding normal tissues [85]. CERS6 overexpression reduced the proliferation of CRC cells and induced apoptosis, whereas CERS2 overexpression increased the proliferation of CRC cells [35]. Regardless of the mechanism, overexpression of CERS2 and CERS6 decreased the viability of CRC cell lines tested [85]. CerS6-generated C16 ceramide was shown to increase apoptosis in colon cancer cells [46].

CERS5-ko mice showed significantly larger colon tumors than CERS5-wt mice [86]. Another study showed that strong CERS5 staining correlated with poor prognosis in patients with CRC [87]. CERS4 and CERS5 were also found to be upregulated in colon cancer prior to apoptosis induction and down-regulated after apoptosis induction in colon cell lines [88].

The importance of ceramide levels in cancer cells was also demonstrated in studies with ceramide analogs such as LCL-30, the cationic water-soluble analog of C16-ceramide. LCL-30 accumulates in cells' mitochondria and induces mitochondrial swelling, decreases membrane potential, caspase activation, and ultimately cell death [89, 90]. The same group also tested its actions in colon carcinoma cell line CT-26 as an *in vivo* model of colorectal cancer, demonstrating that LCL-30 was cytotoxic to CT-26 cells [90].

Adiseshaiah et al. also showed that injection of nanoliposomal C6-ceramide, an autophagy inducer, in combination with vinblastine, decreased tumor growth in comparison to the individual treatments [75]. The authors used the colon cancer xenograft model (LS174T) and showed that the combination treatment resulted in statistically significant suppression of tumor growth compared to a single treatment. The rationale behind the study was that cancer cells might evade anticancer therapy by inducing autophagy, so blocking it should improve therapeutic response.

It is undoubtedly that microenvironment will largely influence cancer cells' fate during their life cycle. Cancer cell progression is associated with tumorigenic M2 macrophages. Ceramide-treated macrophages were shown to induce the switching of macrophage polarization toward the pro-inflammatory M1-phenotype. Ceramide also abolished macrophage-induced epithelial-mesenchymal transition and migration of colorectal cancer cells [91]. Other studies have demonstrated that M1 and M2 macrophages can switch phenotypes and lipids have the potential to modulate their function and phenotypes [92, 93]. Ceramides act as an intracellular second messenger and membrane component [94]. Araujo Junior et al. have demonstrated that ceramide can reduce M2 phenotype and block migration of cancer cells, suggesting that targeting ceramide in the tumor microenvironment could, in theory, reduce tumor progression and potential for metastasis of colon cancer cells [91].

Ceramide is also generated by the hydrolysis of sphingomyelin by SMases—acid, neutral, and alkaline—based on their pH-dependent optimal activity.

The activities of neutral and alkaline SMase were highest in the ascending colon and decreased in the sigmoid colon and rectum, whereas no significant difference

was found for acidic SMase activity at all locations [48]. Markowski et al. also examined the relationship of sphingolipids levels in CRC tissue on tumor localization and documented that, albeit complex and ambiguous, the number of total ceramides was lowest in sigmoid and cecum tumors and the largest in rectal tumors [36]. SMase activity was found to be decreased in colorectal carcinomas, mainly alkaline SMase activity, which results in lowered cellular levels of ceramide. In comparison to surrounding normal tissue, SMase activity in colorectal cancer is reduced by 75%, 50%, and 30% for alkSMase, nSMase, and aSMase, respectively [48].

3.2.2 Pro-S1P metabolic pathways

So, on one side of the balance, we can identify the mechanisms responsible for ceramide raised levels; however, on the other side, we should pay attention to the antagonist mechanisms leading to the degradation of ceramide in detriment to S1P and their transitory metabolites.

Among the five ceramidases identified to date [95], neutral CDase is predominantly expressed in the colon and is involved in the metabolism of dietary sphingolipids [96]. It was shown that inhibition of NCDase induces an increase of ceramide in colon cancer cells, decreasing cell growth and increasing apoptosis [50, 81]. Coant et al. also showed that deletion of NCDase protected mice from the onset and progression of colorectal cancer C16:0 ceramide levels were increased. The inhibition of NCDase leads to inhibition of the WNT/β-catenin pathway [81]. HT 29 colon cancer cells treated with NCDase inhibition were accompanied by decreased survival, increased apoptosis, and autophagy [50]. Animal studies also showed that inhibition of NCDase delayed tumor growth, with increased ceramide and reduced tumor cell proliferation [50]. Taken together, NCDase appears to be an important target for new therapeutic strategies.

Studies in mice have demonstrated that oral administration of plant-type sphingolipids increased colonic Sphingosine-1-phosphate lyase (SPL) levels and reduced S1P levels, cytokine levels, and tumorigenesis, indicating that SPL can prevent transformation and carcinogenesis [53]. These studies suggest that dietary sphingolipids can have a role in colon cancer prevention in opposition to high-fat diets that possibly increase the risk of colorectal cancer. SPL is highly expressed in normal intestinal and colonic epithelium, however, it is downregulated in CRC cells and in early adenomatous lesions of Min mice [54]. SPL expression promotes apoptosis through a cascading mechanism that involves p53, p38, PIDD, and caspase-2; however, it is not clear how this interaction occurs [54]. SPL activity provides an exit route from sphingolipid metabolism via the rapid hydrolysis of S1P. SPL appears to be downregulated at the protein level in colon cancer tissues, and SPL silencing promoted colon carcinogenesis, which occurred via S1P accumulation and/or S1PR signaling [53]. On the contrary, SPL overexpression leads to increased apoptosis through reduced S1P signaling in colon cancer cells [54].

The two isoforms of sphingosine kinase, SPHK1, and SPHK2, utilize sphingosine and generate S1P but have significant differences in subcellular localization and function [51]. Sphingosine kinases (SPHK1 and 2) are overexpressed in many cancers, including colorectal cancer, compared with normal mucosa [97]. The expression levels of SPHK1 and 2 were also high in liver metastases compared with matched normal colon tissues. SPHK1 and SPHK2 are observed in different places within the cell; SPHK1 in the cytosol while SPHK2 was detected in both cytosol and nucleus [97]. SPHKs seem to have a role in promoting the metastatic potential of colorectal cancer

cells [97]. FTY-720, an S1P receptor antagonist, reduces cell migration and invasion and significantly decreases cellular proliferation in all cell lines tested [97].

3.3 Sphingolipids, treatment resistance, and new strategies

5-Fluorouracil (5-FU) is one of the first-line chemotherapy agents' in colorectal cancer and despite its efficacy, drug resistance is still an important limitation. Jung et al. conducted a lipidomic analysis showing that resistance to 5-FU is associated with the up-regulation of sphingomyelin and the down-regulation of CERS [98].

SPHK1 contribution to cetuximab resistance in colorectal cancer was investigated. The authors found overexpressed and overactivated SPHK1 in colorectal cancer cells with intrinsic or acquired resistance to cetuximab [24]. It was also documented that treatment of resistant cells with FTY-720 resulted in resensitization to cetuximab both *in vitro* and *in vivo* [24]. This association could be a new therapeutic strategy to overcome chemotherapy resistance and also a biomarker of interest for cetuximab resistance.

In another study involving SPHK2, the authors found that using ABC294649, a novel SPHK2 inhibitor, resulted in growth inhibition and apoptosis of CRC cells, with S1P depletion and ceramide incrementation. Also, exogenously-added S1P inhibited ABC294640 cell effects. The authors also described that ABC294649 sensitized 5-FU and cisplatin-mediated anti-HT-29 cell activity. This agent could be an important anti-CRC weapon, and it is also available in an oral formulation [58]. Xun et al. demonstrated in HT-29 cell lines that SphK2 inhibition (ABC294640) resulted in S1P depletion and ceramide incensement with consequent cell lethality. Oral administration dramatically inhibited H-29 xenograft growth in nude mice [58].

SphK inactivation induces the accumulation of S1P precursors, including sphingosine and ceramide, causing cell apoptosis and growth arrest [99].

Activity in primary cancer cells was also tested. SphK2 expression was different between patients, however, ABC294640 activity was negatively associated with SphK2 expression level [58].

Glucosylceramide synthase (GCS), a ceramide-metabolizing enzyme, has been demonstrated to be overexpressed in CRC tissues compared with non-CRC tissues. Wang et al. documented that high-expression GCS patients were associated with significantly higher lymph node metastasis than the low CGS expression group [63].

GCS has been associated with several studies that documented its role in chemotherapy resistance [63, 100]. Oxaliplatin-resistant cells demonstrated increased expression of GCS protein compared to the parental cell line, with increased levels of glucosylceramide (GlcCer) [100]. Madigan et al. also showed that inhibition of GCS expression resulted in the reduction of ClcCer levels with restored sensitivity to oxaliplatin. Oxaliplatin-resistant CRC cells also expressed lower ceramide levels compared to parental cells. In fact, the conversion of ceramide to glucosylceramide by GCS represents an essential mechanism for limiting ceramide accumulation [101]. It was also shown that the rate of GCS was higher in patients receiving neoadjuvant chemotherapy than in non-CRC tissues, raising the possibility that chemotherapy drugs might induce the high expression of GCS and increase the risk of MDR [63]. The authors hypothesized that oxaliplatin treatment might result in reduced ceramide levels compared to oxaliplatin-sensitive cells. C16-ceramide was the only species to differ significantly between the two cell lines. Higher sphingomyelin levels were found in the positive nodes of colorectal cancer patients compared to the negative lymph nodes [102].

In recent years, a few new pharmacologic strategies have been used in laboratory and clinical trials. Fenretinide (preclinical; reduces de novo synthesis with dihydroceramide accumulation), Safingol (association with irinotecan, preclinical; SPHK1 inhibitor), Ceramide nanoliposomes (association with tamoxifen, preclinical; apoptosis promoter by ceramide accumulation), α-GalCer (preclinical; α- galactosylceramide-pulsed antigen-presenting cells), and Fingolimod (association with sphingosine and cetuximab, preclinical; functional antagonist of the sphingosine-1-phosphate receptor (S1PR) and structural analog of sphingosine) [60, 103] are the most important in colorectal cancer with exciting and promising results.

4. In summary

Sphingolipids are structural molecules of cell membranes with an essential role in barrier and fluidity functions. They have been implicated in many physiologic and pathologic processes, such as cell growth, cell death, adhesion, proliferation, stress, inflammatory responses, differentiation, migration, invasion, and/or metastasis.

The sphingolipids play an essential role in cancer biology and influence treatment response and aggressiveness. It also happens in colorectal cancer and may be interesting in developing an individualized treatment plan for LARC.

Nevertheless, the molecule's action interpretation is complicated, given the complexity of sphingolipid's metabolism with several activations and counter-regulation pathways. In addition, there are isoforms whose action is different depending on the location in the cell and the type of tissues in which they occur. Finally, the balance among ceramides has also essential for the activity response.

However, we can state that in general terms, there are two central bioactive lipids, ceramides and sphingosine-1-phosphate (S1P), which have opposing roles in regulating cancer cell death and survival. Ceramides have been shown to mediate cell cycle arrest and cell death in response to cell stress. Also, the equilibrium between ceramides of various chain lengths is crucial for cell fate. On the other hand, S1P has been shown to promote cell survival and proliferation.

Thus, the increase in specific ceramides in the tumor may correspond to a lower aggressiveness or effective response to the therapy instituted. In comparison, the rise in S1P in the tumor will correspond to a greater aggressiveness of tumor resistance to the treatment.

In this perspective, the measurement of ceramides and S1P may be of interest to assess the aggressiveness of a particular tumor. Nevertheless, on the other hand, we can try to interfere with the amount of these elements present in the tumor to modify tumor resistance to conventional therapy.

From published studies, it appears that sphingolipids' metabolism in tumor tissue is unsettled in colorectal cancer.

Ceramides and their proportion are different in plasma of patients with CRC and tumor tissue compared with plasma and tissue control levels. On the other hand, plasma ceramide concentration is not directly related to ceramide concentration in tumor tissue.

The knowledge gathered in the past decade can lead us to new ways of treating CCR patients, trying to overcome treatment resistance, and, in the end, achieving higher response rates and improved global life expectancy.

In conclusion, the knowledge of tumor sphingolipids metabolism may be essential in colorectal cancer treatment. Unfortunately, the studies about this issue are small and few. Therefore, investigation in this area is needed.

Conflict of interest

The authors declare no conflict of interest.

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