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Sphingolipid metabolism enzymes as validated targets for overcoming tumor therapeutic resistance

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Abstract. This work analyzes the mechanisms of tumor therapeutic resistance mediated by metabolic adaptations in the sphingolipid pathway. It describes the concept of the "sphingolipid rheostat," where a shift from pro-apoptotic ceramide to pro-survival sphingosine-1-phosphate (S1P) promotes cell survival under stress. The review highlights the key enzymes driving this shift—glucosylceramide synthase (GCS), acid ceramidase (AC), and sphingosine kinase 1 (SPHK1)—as validated therapeutic targets. It is demonstrated that pharmacological inhibition of these enzymes can restore tumor sensitivity to chemotherapy, targeted drugs, and immunotherapy, offering a promising strategy for overcoming multidrug resistance.
Keywords: *sphingolipid metabolism, therapeutic resistance, ceramide, sphingosine-1-phosphate, glucosylceramide synthase, sphingosine kinase, acid ceramidase, cancer therapy.*

Introduction. Therapeutic resistance remains one of the major obstacles to effective cancer treatment and is a key factor leading to disease recurrence [3]. Numerous studies demonstrate that resistance formation is underpinned not only by the genetic heterogeneity of tumors but also by their profound metabolic adaptation [3]. Among the metabolic pathways playing a critical role, sphingolipid metabolism stands out. Sphingolipids are a class of bioactive lipids that act not merely as structural membrane components but as key messengers controlling fundamental cellular decisions: proliferation, differentiation, apoptosis, and stress response [1, 2, 3].

Central to understanding their role in oncology is the concept of the "sphingolipid rheostat" [1]. This model

describes the dynamic balance between bioactive sphingolipids possessing opposing functions [2]. On one hand, ceramide functions as a pro-apoptotic signal; its intracellular accumulation in response to cellular stress caused by chemotherapy or radiation is one of the primary mechanisms of their therapeutic action [2]. On the other hand, sphingosine-1-phosphate (S1P) acts as an oncogenic mediator stimulating proliferation, migration, angiogenesis, and preventing apoptosis. Such mediators provoke cell division and increase their survival probability under given conditions [1, 2, 3].

Therapeutic resistance develops when tumor cells adapt by "shifting" sphingolipid metabolism towards survival, actively enhancing the metabolism (catabolism) of ceramide and its conversion into pro-survival lipids [2, 3]. Enzymes controlling this metabolic shift—specifically glucosylceramide synthase (GCS), sphingosine kinases (SPHK1/2), and acid ceramidase (AC)—often demonstrate overexpression in resistant tumor phenotypes [1, 3, 4, 6]. Consequently, these enzymes are validated therapeutic targets, the pharmacological inhibition of which is capable of disrupting the tumor's protective metabolic pathways and restoring its sensitivity to standard treatment methods [4, 5, 3, 6].

Sphingolipid Network and Resistance Mechanisms. Sphingolipid metabolism is a complex network centered around ceramide [2, 3]. Its level is decisive for cell fate. Therapeutically significant enzymes, such as sphingomyelinase (SMase), generate ceramide from sphingomyelin in response to stress induced by chemotherapy, which triggers apoptosis [2, 3]. Thus, ceramide accumulation represents a pro-apoptotic pathway [2].

However, tumor cells develop resistance through the hyperactivation of enzymes that catabolize and "detoxify" ceramide, shifting the balance towards survival [3]. Key enzymes contributing to such resistance include:

- Glucosylceramide synthase (GCS), which converts ceramide into non-toxic glucosylceramide (GlcCer), additionally activating multidrug resistance (MDR) mechanisms [1, 3, 4, 8].
- Acid ceramidase (AC), which hydrolyzes ceramide to sphingosine, lowering its level while simultaneously providing a substrate for the subsequent enzyme [3, 6, 9].
- Sphingosine kinase 1 (SPHK1), which utilizes this sphingosine to generate a potent survival signal—sphingosine-

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1-phosphate (S1P) [1, 2, 5, 7].

Thus, the sphingolipid balance is essentially a dynamic competition between therapeutic ceramide generation (via SMase) and its pro-resistant catabolism (via GCS, AC, and SPHK1) [1, 2].

The Pro-Apoptotic Pathway: Ceramide as a Therapy Mediator. Numerous standard antitumor agents, including doxorubicin, paclitaxel, as well as radiation therapy, do not always kill cells directly; their efficacy is mediated by the induction of cellular stress, which activates ceramide generation [2, 3]. Upon accumulation, ceramide induces cell death through several mechanisms, the most important being the mitochondrial pathway [2]. It is capable of self-organizing within lipid rafts on the outer mitochondrial membrane, forming stable "ceramide channels" [2]. These structures facilitate the oligomerization of pro-apoptotic BCL-2 family proteins (e.g., BAX and BAK), leading to mitochondrial outer membrane permeabilization (MOMP). This, in turn, causes the release of cytochrome C into the cytoplasm, activation of the caspase cascade, and irreversible apoptosis [2]. Furthermore, recent studies link ceramide accumulation to the induction of alternative cell death pathways, specifically ferroptosis, which expands its role as a sensitizer to targeted drugs such as sorafenib [3].

The Pro-Survival / Resistant Pathway: Enzymatic "Escape". Tumor cells survive because they have developed potent mechanisms to prevent lethal ceramide accumulation, which underlies their drug resistance. They achieve this through constitutive or induced hyperactivation of enzymes that catabolize ceramide [3].

The first line of defense is ceramide hydrolysis. Acid ceramidase (AC), often overexpressed in melanoma, prostate cancer, and multiple myeloma, cleaves ceramide to sphingosine [3, 6]. This inherently lowers pro-apoptotic pressure. AC overexpression correlates with acquired resistance to 5-fluorouracil (5-FU) in colorectal cancer [9] and to proteasome inhibitors in multiple myeloma [6].

However, subsequently, sphingosine kinase 1 (SPHK1) converts the resulting sphingosine into S1P, completing the metabolic "switching" of the rheostat from death to survival [1, 2]. SPHK1 overexpression is one of the most common features of aggressive and resistant tumors [1, 3]. S1P acts as an extracellular ligand for its receptors (S1PRs), activating oncogenic signaling pathways, including PI3K/AKT,

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NF-κB, and STAT3 [1, 7]. Recent studies confirm that the SPHK1/S1PR3/STAT3 axis is a key mechanism of resistance to sunitinib in renal cell carcinoma [7]. Moreover, SPHK1 promotes resistance to immunotherapy (anti-PD-1) by creating an immunosuppressive microenvironment [5].

The second, parallel "escape" pathway is ceramide glycosylation. Glucosylceramide synthase (GCS), encoded by the UGCG gene, catalyzes the conversion of ceramide into non-toxic glucosylceramide (GlcCer) [1, 3]. GCS overexpression is associated not only with reduced ceramide levels but also with the development of the multidrug resistance (MDR) phenotype [3]. The mechanism lies in the fact that the accumulation of GlcCer and more complex glycosphingolipids (GCS products) activates signaling pathways (e.g., PI3K/AKT/GSK-3β/β-catenin) [8], which lead to enhanced transcription of the ABCB1 gene [3]. This gene encodes P-glycoprotein (P-gp)—a potent ATP-dependent efflux pump. This "molecular pump" actively recognizes and extrudes a broad spectrum of chemotherapeutic drugs (including doxorubicin, paclitaxel, and vincristine) out of the cell before they can take effect [3, 8]. Beyond classical chemotherapy, GCS is also linked to resistance to modern targeted drugs, such as the EGFR inhibitor osimertinib [4].

Balance Disruption as a Resistance Mechanism. Thus, in resistant tumors, the "sphingolipid rheostat" is not merely shifted; it is dynamically utilized to counteract treatment [2]. This forms a "vicious cycle":

1. Therapy (e.g., doxorubicin) induces stress, leading to a transient increase in pro-apoptotic ceramide levels [2, 3].
2. In response to this stress, adapted tumor cells upregulate the expression or activity of "escape" enzymes such as GCS, AC, and SPHK1 [3, 9].
3. These enzymes rapidly catabolize therapeutic ceramide, converting it into GlcCer (activating P-gp) [8] or S1P (activating STAT3 and AKT) [7].
4. As a result, the pro-apoptotic signal is not only neutralized but transformed into a potent pro-survival signal [1, 2].

This metabolic reprogramming is a fundamental non-mutational mechanism allowing tumor cells to survive therapeutic impact and form acquired resistance [2, 3].

Clinical Implications and Therapeutic Targeting. Dysregulation of sphingolipid pathway enzymes is not merely

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a laboratory phenomenon; it has direct clinical correlations and serves as the basis for validating these enzymes as therapeutic targets [1, 2, 3].

Target Validation. Numerous clinical studies confirm that the expression levels of these enzymes in patients correlate with prognosis and treatment response.

- SPHK1: Overexpression of sphingosine kinase 1 (SPHK1) is associated with poor survival and treatment resistance in many types of solid tumors, including colorectal cancer, breast cancer, and renal cell carcinoma [1, 3, 7]. It is also a proven factor contributing to resistance to immunotherapy (anti-PD-1) in melanoma [5].
- GCS: Similarly, high levels of glucosylceramide synthase (GCS) correlate with the multidrug resistance (MDR) phenotype and poor prognosis in breast and colorectal cancer [3, 8]. Its expression is also a mechanism of acquired resistance to targeted therapy with EGFR inhibitors [4].
- AC: Overexpression of acid ceramidase (AC) is linked to resistance to 5-fluorouracil in colorectal cancer [9] and to proteasome inhibitors in multiple myeloma [6].

Treatment Strategies. The direct consequence of validating these enzymes is the development of strategies aimed at their pharmacological inhibition. The primary therapeutic logic is not necessarily to use these inhibitors as monotherapy, but rather to use them as sensitizers to overcome resistance to standard treatment methods [2, 3].

A range of selective inhibitors targeting "escape" enzymes has been developed. These include SPHK inhibitors such as FTY720 (Fingolimod) and ABC294640 (Opaganib) [2, 3], as well as GCS inhibitors (e.g., PDMP and PPMP) [3, 4], and AC inhibitors (e.g., Ceranib-2) [3, 6].

These inhibitors block the metabolic "escape" pathways utilized by the tumor to neutralize therapeutic stress. When a resistant cell undergoing chemotherapy (e.g., with doxorubicin) simultaneously receives a GCS or SPHK1 inhibitor, it loses the ability to catabolize toxic ceramide [2, 4]. The ceramide generated by therapy can no longer be "detoxified" via conversion into GlcCer or S1P. It accumulates within the cell, forcibly shifting the "sphingolipid rheostat" back to a pro-apoptotic state [2]. Thus, inhibitors of sphingolipid pathway enzymes restore tumor sensitivity to chemo-, radio-, and targeted therapy [4, 5, 6, 7]. The clinical significance of this approach is supported by the fact that several inhibitors, notably ABC294640 (Opaganib),

are already undergoing Phase II clinical trials for the treatment of refractory tumors [2, 3].

Conclusions. Dominant theories of therapeutic resistance focusing on genetic mutations in drug targets are incomplete in explaining the full complexity of this phenomenon [3]. Current data convincingly demonstrate that the metabolic adaptation of tumor cells is a critical, often non-mutational axis of resistance pathogenesis [2, 3]. In this context, the dysregulation of sphingolipid metabolism plays a pivotal role [1].

Disruption of the "sphingolipid rheostat" balance—namely the shift in the ratio from pro-apoptotic ceramide to pro-survival S1P—is a fundamental tumor defense mechanism [1, 2]. This shift, driven by the hyperactivation of "escape" enzymes (GCS, SPHK1, and AC), forms a state of chronic "metabolic exhaustion" of therapy, wherein the pro-apoptotic signal induced by treatment is not only neutralized but actively transformed into a survival signal [2, 7, 8].

Therefore, these enzymes are validated therapeutic targets. Combination therapy integrating standard cytostatics [3, 4, 8], targeted drugs [3, 7], or immune checkpoint inhibitors [5] with selective pharmacological inhibitors of GCS, SPHK1, or AC represents an extremely promising approach for sensitizing refractory tumors and overcoming resistance [1, 6]. Furthermore, the development of clinical protocols to measure expression levels of these enzymes or to quantify metabolite ratios (ceramide/S1P) in patients may serve as powerful prognostic and predictive biomarkers for stratification and the prescription of personalized treatment [1, 2].

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