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Review

Tamoxifen regulation of sphingolipid metabolism—Therapeutic implications

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ABSTRACT

Tamoxifen, a triphenylethylene antiestrogen and one of the first-line endocrine therapies used to treat estrogen receptor-positive breast cancer, has a number of interesting, off-target effects, and among these is the inhibition of sphingolipid metabolism. More specifically, tamoxifen inhibits ceramide glycosylation, and enzymatic step that can adventitiously support the influential tumor-suppressor properties of ceramide, the aliphatic backbone of sphingolipids. Additionally, tamoxifen and metabolites *N*-desmethyltamoxifen and 4-hydroxytamoxifen, have been shown to inhibit ceramide hydrolysis by the enzyme acid ceramidase. This particular intervention slows ceramide destruction and thereby depresses formation of sphingosine 1-phosphate, a mitogenic sphingolipid with cancer growth-promoting properties. As ceramide-centric therapies are becoming appealing clinical interventions in the treatment of cancer, agents like tamoxifen that can retard the generation of mitogenic sphingolipids *and* buffer ceramide clearance via inhibition of glycosylation, take on new importance. In this review, we present an abridged, lay introduction to sphingolipid metabolism, briefly chronicle tamoxifen's history in the clinic, examine studies that demonstrate the impact of triphenylethylenes on sphingolipid metabolism in cancer cells, and canvass works relevant to the use of tamoxifen as adjuvant to drive ceramide-centric therapies in cancer treatment. The objective is to inform the readership of what could be a novel, off-label indication of tamoxifen and structurally-related triphenylethylenes, an indication divorced from estrogen receptor status and one with application in drug resistance.

1. Introduction

The antiestrogen tamoxifen demonstrates a myriad of non-genomic activities; curiously among these is inhibition of sphingolipid (SL) metabolism at ceramide glycosylation and at ceramide hydrolysis. This is principally noteworthy because sphingolipids are remarkably active lipids that orchestrate events that regulate cancer cell death as well as proliferation, ceramide more specifically, a pro-death player, and sphingosine 1-phosphate (S1P), a pro-life manager (Fig. 1). In survival mode, cancer cells have or acquire enzymatic operatives to suppress ceramide potency, glycosylation by glucosylceramide synthase (GCS) producing glucosylceramide (GC), and hydrolysis by acid ceramidase (AC), ceramide destruction. Destruction however is a double-edged sword that boosts mitogenic potential through phosphorylation of sphingosine by sphingosine kinase (SphK). Tamoxifen, tamoxifen metabolites, and related triphenylethylenes (TPE's), as inhibitors of ceramide glycosylation and hydrolysis, can thus govern ceramide-regulated apoptotic cell death by effectively undoing ceramide resistance mechanisms and thwarting downstream mitogenicity. In the scheme of cancer therapeutics, whether ceramide-generating agents are administered or whether cell-deliverable ceramides are used as anti-cancer agents, use of TPE's in ceramide-centric drug regimens presents an interesting direction we believe is worthy of exploitation. This review will focus on the role of TPE's as regulators of SL metabolism and as adjuvants to ceramide-based cancer therapies.

2. Sphingolipid metabolism in a nutshell

Sphingolipids are a class of nitrogen-containing lipids that harbor ceramide, a tumor suppressor lipid [1–3], as the aliphatic backbone (Fig. 1). If SL's contain sugars, they are termed glycosphingolipids. SL's serve structural roles in cellular membranes, and ceramide in particular has wide impact on cell function via mosaic signaling cascades [2]. By and large, tumor cells are protected from ceramide's apoptosis-inducing, deleterious effects by constructive metabolic steps that

Abbreviations: SL, sphingolipid; S1P, sphingosine 1-phosphate; GCS, glucosylceramide synthase; GC, glucosylceramide; AC, ceramidase; SphK, sphingosine kinase; PPMP, D-*threo*-1-phenyl-2-hexadecanoylamino-3-morpholino-1-propanol; PDMP, D-*threo*-1-phenyl-2-decanoylamino-3-morphomolino-1-propanol; TPE's, triphenylethylenes; DMT, *N*-desmethyltamoxifen; SM, sphingomyelin; SERM, selective estrogen receptor modulator; ACAT, Acyl-CoA:cholesterol acyl transferase; DTIC, dacarbazine; BCNU, carmustine; ABC, ATP binding cassette; P-gp, P-glycoprotein; MRP, multidrug resistance protein; NB-DNJ, N-butyl-deoxynojirimycin; 4-HPR, *N*-(4-hydroxylphenyl) retinamide; ER, endoplasmic reticulum; LC, lactosylceramide; LCS, lactosylceramide synthase; SPT, serine palmitoyltransferase; AML, acute myeloid leukemia

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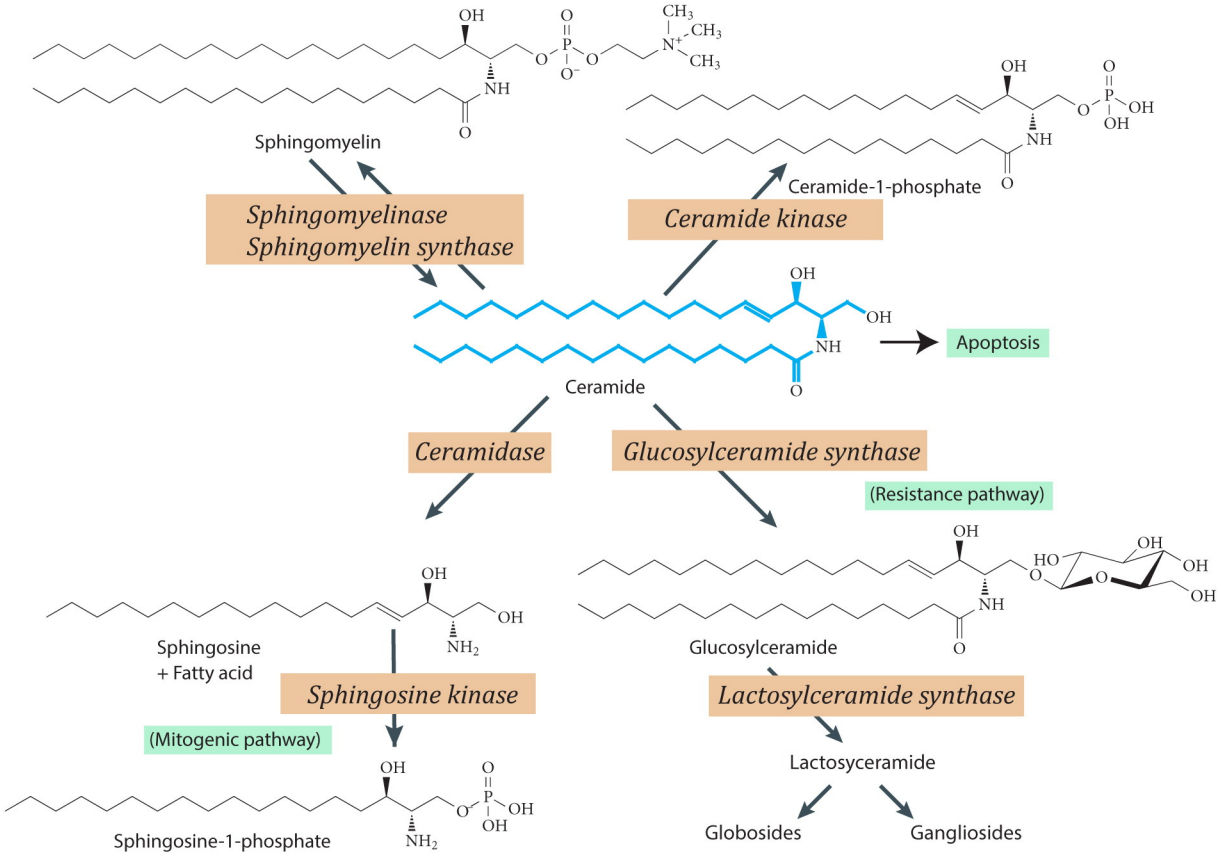

Fig. 1. Anabolic and catabolic routes of ceramide metabolism. Ceramide, shown in blue, the aliphatic backbone of sphingolipids, can be converted to sphingomyelin, ceramide 1-phosphate, and glucosylceramide. Ceramide, a tumor suppressor, induces apoptosis. Glycosylation (conversion to GC) is a prominent metabolic pathway in multidrug resistant cancer cells; glycosylation as well leads to ceramide resistance. If ceramide is hydrolyzed by ceramidase, sphingosine and free fatty acid are generated. The sphingosine can be metabolized to sphingosine 1-phosphate, a mitogenic entity, by sphingosine kinase. Various points in ceramide metabolism can be activated or inhibited, providing a useful strategy for studying ceramide-related events. GC, glucosylceramide. Note: arrows to designate several of the back-reactions are not included in this figure; for example, beta-glucocerebrosidase, also known as D-glucosyl-*N*-acylsphingosine glucohydrolase, which catalyzes cleavage by hydrolysis of the beta-glucosidic linkiage of glucosylceramide.

produce a wide variety of noteworthy lipids such as sphingomyelin (SM), GC, precursor of higher cerebrosides, globosides, and gangliosides, and galactosylceramides, precursors of sulfatides. Of particular interest here is ceramide glycosylation, the major metabolic pathway utilized by multidrug resistant cancer cells to facilitate ceramide clearance [4–6]. Whereas ceramide is a powerful tumor censor, the glycosylated product, GC, formed by the action of GCS (Ceramide: UDP-Glc Glucosyltransferase), is ineffectual in this realm. Upregulated ceramide glycosylation is a mechanism of ceramide resistance in cancer cells [7–11]. Also relevant is ceramide hydrolysis, specifically by acid ceramidase, another sentinel enzyme regulator of cancer cell growth [12–14]. Similar with GCS, AC thwarts the tumor-killing properties of ceramide via hydrolysis, producing fatty acid and sphingosine, the latter a substrate for Sphk, the enzyme catalyzing production of S1P, a cancer cell mitogen [15,16].

Ceramide can be synthesized de novo via enzymatic reactions that open with condensation of serine and palmitoyl-CoA to form sphinganine (Fig. 2); this reaction is catalyzed by serine palmitoyltransferase (SPT). The process concludes with an acylation step conducted by dihydroceramide synthase to generate dihydroceramide; this is followed by insertion of a 4,5-*trans* double in the sphingoid base moiety. A myriad of anticancer agents stimulate the de novo pathway and as well provoke degradation of SM to produce ceramide [17]. Both the de novo and the sphingomyelinase pathways are crucial therapeutic targets for the generation of ceramide in cancer cells, and whereas ceramide produced by these pathways can elicit apoptotic responses, anticancer efficacy can be limited by ceramide metabolic clearance (Fig. 1).

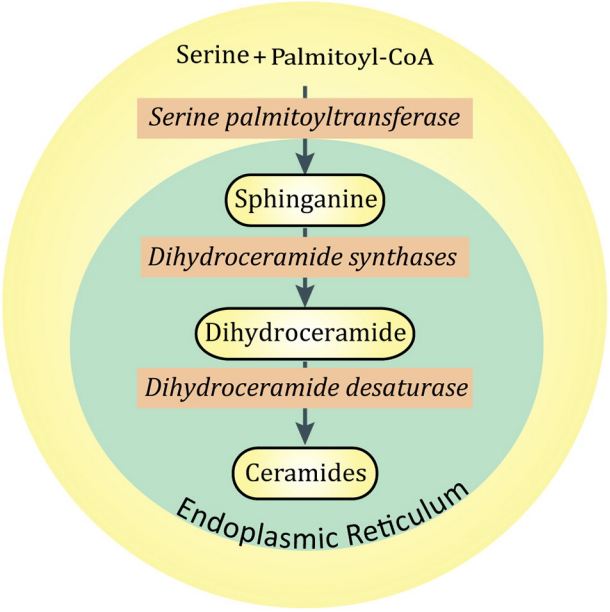

Fig. 2. De novo pathway enzymes for production of ceramide. Ceramide synthesis is initiated by serine palmitoyltransferase and culminates with addition of a 4,5-*trans* double bond in dihydroceramide, catalyzed by dihydroceramide desaturase. Ceramide synthases, which number 6, are the subject of engaged investigations due to their selectivity for an array of fatty acyl-CoAs, giving rise to a myriad of molecular species of ceramides with distinct biological properties. Enzymes of the de novo pathway are shown in italics.