ORIGINAL RESEARCH PAPER

Volume-8 | Issue-8 | August - 2019 | PRINT ISSN No. 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

CERAMIDE –CENTRAL HUB OF BIOACTIVE-SIGNALLING MOLECULES OF SPHINGOLIPID METABOLISM.

Biochemistry

Prof Pramila kumari Mishra

HOD, Department of Biochemistry, M. K. C. G. Medical college, Berhampur, Odisha

ABSTRACT

Ceramide like other lipid second messengers in signal transduction are produced rapidly and transiently in response to specific stimuli in order to target specific proteins under all conditions of cellular stress by multiplicity of activators.Ceramide the key intermediate in biosynthesis of complex sphingolipids is the central hub of bioactive signalling molecules of SL family, sphingosine(sph),sphingosine-1-phosphate (SIP) & ceramide1-phosphate (CIP) in Spingolipid metabolism. Ceramide with sphingosine back bone is generated from diverse pathways, denovo synthesis & hydrolysis of SM and cerebrosides. Ceramide formed is transported to golgi by a transfer protein(CERT) where sphingomylin,(SM) and glucosylceramide (Glucer) are formed.(1) Spingolipids in membrane long assumed to serve only structural roles ,are also regulators of cell growth ,death, senescence, adhesion, migration, inflammation and intracellular trafficking.

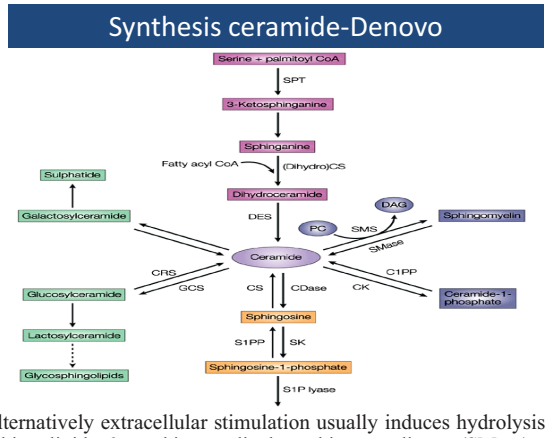
KEYWORDS

Spingolipids, sphingomylin, ceramide, sphingosine.

INTRODUCTION-

Sphingolipids (SL), a major class of lipids are essential constituents of eukaryotic cell membrane was first discovered by J.L.N.Thudicum in 1876 & for a long time was considered to play structural roles in membrane formation. Intensive research on SL metabolism & function has revealed that metabolites of SL family, ceramide(cer) sphingosine (sph), sphingosine-1-phosphate (SIP) & ceramide1-phosphate (CIP) as bioactive signalling molecules. Ceramide is produced by palmitoyl transferase mediated interaction of serine & palmitoyl COA & then a series of metabolic reactions. Sphingolipid contains sphingosine, an amide linked long chain fatty acid & one of several polar head groups. The polar head group defines various sphingolipid classes with hydroxyl-group in ceramide such as phosphorylcholine in sphingomylin and carbohydrate in various glycosphingolipids.

FIG-1

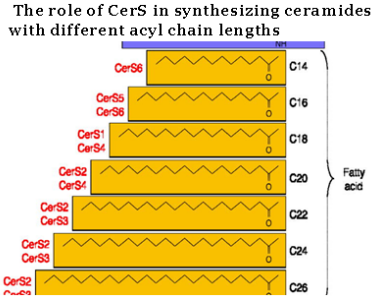


Alternatively extracellular stimulation usually induces hydrolysis of sphingolipids & sphingomylin by sphingomyelinase (SMase) and cerebrosides including galactosylceramide and glucosylceramide by cerebrosidase. In order to maintain homeostasis of sphingolipid metabolism, Ceramide is subsequently metabolized by ceramide kinase to generate Ceramide -1-phosphate (C1P) & by ceramidase to generate sphingosine, which is further phosphorylated to S1P by sphingosine kinase. Again dephosrylation occurs by using specific phosphatases(C1P-phosphatase & S1P-phosphatase). Further ceramide can also be produced from sphingosine by ceramide synthase (2,3).

However Ceramide synthase at the centre of SPL metabolism regulates denovo synthesis & recycling of free sphingosine produced from preformed SPL in salvage pathway.Six isoforms of cers produced with characterstic acyl chain lenth, encoded by 6 genes as longevity –assurance homolog (LASSI-6) .Six mammalian genes that encode Ceramide synthase have recently been cloned.

Cer synthase (CerS1-6) isoform shows substrate preference for specific chain length fatty acyl CoAs. CerS1, for example,shows significant preference for C18:0 FA CoA, where as CerS5 and CerS6 preferably catalyze the acylation of dihydro Sph with myristoyl-coA, palmitoyl-coA, and stearoyl-CoA compared to very long-chain FA CoAs (4,5).

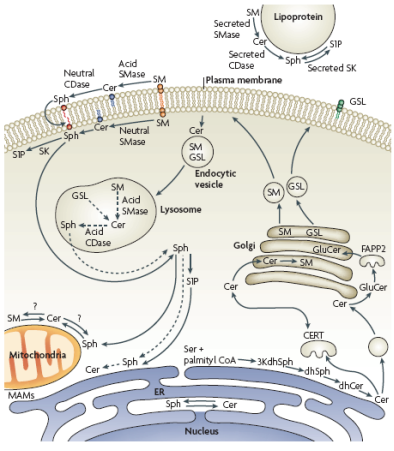
FIG-2



SM is concentrated in the outer leaflet of plasma membrane & provides a barrier to the extracellular environment. SM serves as a reservoir for ceramide . The overall level of ceramide in a cell is a balance between the need for sphingosine & its derivatives such as S1P & SM.

compartmentalization and regulation of bioactive SLS

FIG-3



Understanding the functions of SLs requires insight into the specific subcellular localization of the enzymes involved in the SL pathway. Enzymatic reactions in sphingolipid metabolism are distributed throughout different cellular compartments (6). The initial step of sphingolipid *de novo* synthesis leading to ceramide formation takes place on the cytosolic surface of the endoplasmic reticulum (ER) and possibly on ER associated membranes, such as the perinuclear

membrane and mitochondria-associated membranes (MAMs) .Ceramide formed in this compartment is transported to the Golgi, which is the site of synthesis of sphingomyelin and glucosylceramide (GlcCer). The Cer transport to the Golgi occurs either through the action of the Cer transfer protein CERT, which delivers Cer for SM synthesis or through vesicular transport which delivers Cer for the synthesis of GlcCer. The transfer of GlcCer for glycosphingolipid (GSL) synthesis requires the action of the transport protein FAPP2. GlcCer appears to be synthesized on the cytosolic side of the Golgi, and needs to flip to the luminal side of the Golgi for the synthesis of complex GSLs (gangliosides) . This mechanism is supported by the ABC transporter, P-glycoprotein (also known as MDR1) (7)

Subsequently, SM and complex GSLs are delivered to the plasma membrane by vesicular transport. Acid sphingomyelinase (Acid Smase), present in the outer membrane leaflet or neutral sphingomyelinase (neutral SMase), present in the inner leaflet of the bilayer can metabolize SM to Cer and other bioactive lipids (sphingosine, S1P)(6).

From the plasma membrane, SM and GSL may recirculated through the endosomal pathway and reach the lysosomal compartment, where they are degraded by acid SMase and glucosidases to form Cer. Cer is then hydrolysed by acid ceramidase (CDase) to form sphingosine (Sph). Due to its ionizable positive charge,the salvaged Sph is able to leave the lysosome and shows adequate solubility in the cytosol to move between membranes,including the ER, where it would be available where it would be recycled into Cer. This recycling of sphingosine from the catabolism of complex sphingolipids (SM and GSLs) is termed the "salvage pathway" (9). In fact, exogenous C6-ceramide is subject to deacylation by ceramidase, releasing free sphingosine which in turn undergoes reacylation in a ceramide synthase-dependent manner (10).

It is also important to realize that the subcellular localization of enzymes of SL metabolism is a key determinant of site of action of bioactive SLs. SLs are either hydrophobic or amphipathic molecules that have hydrophobic as well as hydrophilic attributes. Hence, it is not surprising that these molecules are mostly integral components of biological membranes and show little movement between membranes, unless acted upon by specific transport mechanisms (such as Cer transfer protein or FAPP2).

SLS AS BIOACTIVE MOLECULES

Bioactive SLs regulate several downstream targets that mediate their various effects on cell function. The cellular levels of the various bioactive SLs exhibit great differences. Concentrations of Cer, Sph, and S1P differ approximately by an order of magnitude, with Cer presenting the highest and S1P the lowest level. A small change in Cer can therefore drastically increase the levels of Sph or S1P.

Cer and Sph are reported to act as tumor-suppressor lipids involved not only in intracellular but also in extracellular processes. Cer signaling has been intimately involved in the regulation of cell growth, differentiation (11), senescence (12), necrosis (13), proliferation (14), and apoptosis (15). Cer functions, at least in part, by activating protein phosphatases. It has also been shown that Cer may regulate protein kinase C (PKC) ,raf-1 , and the kinase-suppressor of Ras, significantly changing the level of phosphorylation of various key substrates. Another binding target for Cer is the cellular protease cathepsin D, which may mediate the actions of lysosomally generated Cer .

Sph has been connected with cellular processes such as inducing cell cycle arrest and apoptosis by modulation of protein kinases and other signaling pathways. It has roles in regulating the actin cytoskeleton and endocytosis and has been shown to inhibit PKC,regulating endocytosis, cell cycle arrest, and protein synthesis(16,17)

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S1P can be seen as a tumor-promoting lipid involved in the regulation of proliferation, cell growth, cell survival, cell migration, inflammation, angiogenesis, vasculogenesis, and resistance to apoptotic cell death, and therefore shows antagonizing effects to those of Cer. S1P binds to G protein-coupled receptors (S1P receptors) on the cell surface, thus regulating diverse G proteins and subsequently various intracellular signaling pathways.

Recent studies have shown the bioactive SLs such as C1P, GlcCer, lyso-SM, and dhCer. Metabolism and biological function of S1P and C1P have recently been reviewed .C1P has been implicated in playing roles in inflammation and vesicular trafficking. It mediates the activation of phospholipase A2 and the release of arachidonic acid in response to interleukin-b. GlcCer has be shown to be involved in post-Golgi trafficking and in drug resistance.

CERAMIDE GENERATION

The dynamic regulation for ceramide generation and metabolism is critical for cellular responses to extracellular stimuli.

In recent years, substantial evidence has demonstrated that Cer generation pathways such as the SMase and de novo pathway are activated by inducers of apoptosis or growth arrest.

Endogenous Cer is produced in response to various stimuli such as cytokines, heat stress (4), UV radiation, hypoxia/ reperfusion, lipopolysaccharides chemotherapeutic agents, and other miscellaneous agents (6). TNF-a and IL-1 have been shown to act on the de novo pathway as well as on nSMase (2). On the other hand, aSMase is induced by various stress stimuli, such as UV and ionizing radiation, ligation of death receptors, and chemotherapeutic agents such as platinum, paclitaxel, and histone deacetylase inhibitors.

Recent studies also implicate an activation of aSMase by reactive oxygen species and nitrosative stress. Activation of aSMase in response to phorbol esters and to UV radiation has been shown to lead to an increase in Cer formation through the salvage (or recycling) pathway, i.e., by inducing formation of Cer, then Sph, which is recycled back to Cer (3).

SK is activated by various growth factors such as epidermal growth factor, platelet-derived growth factor, and proinflammatory cytokines such as TNF-a and IL-1. However this reaction is part of a complex signaling cascade dependent on upstream regulators such as PKC, phospholipaseD,and the extracellular signal-regulated kinase mitogen-activated protein kinases.The change in the level of the product S1P subsequently regulates cell viability and inflammatory responses.

CONCLUSIONS

The individual species of ceramide functions need more investigation. Many useful analytical and molecular tools that now enable extensive research in metabolic, topologic, structural, and functional aspects of Cer generations.

One bioactive SL metabolically transformed into another bioactive molecule, often with counter functions (e.g., Cer and S1P). So the individual regulated pathways, & also the complex network of SL metabolism,need to be studied to understand and integrate SL function and regulation,determining the compartmentalization of both the involved enzymes and their substrates and products.

Enzymes of SL metabolism have now been cloned and characterized, and several SL specific antibodies have been generated, which can provide the possibility and the tools to study the SL enzyme actions.

The application of mass spectrometry also allows specific qualitative and quantitative analysis of endogenous SL.

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