CHAPTER 8

Ceramide-1-Phosphate in Cell Survival and Inflammatory Signaling

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Abstract

A n important metabolite of ceramide is ceramide-1-phosphate (C1P). This lipid second messenger was first demonstrated to be mitogenic for fibroblasts and macrophages and later shown to have antiapoptotic properties. C1P is also an important mediator of the inflammatory response, by stimulating the release of arachidonic acid through activation of group IVA cytosolic phospholipase A₂, the initial rate-limiting step of eicosanoid biosynthesis. C1P is formed from ceramide by the action of a specific ceramide kinase (CerK), which is distinct from the sphingosine kinases that synthesize sphingosine-1-phosphate. CerK is specific for natural ceramides with the erythro configuration in the base component and esterified to long-chain fatty acids. CerK can be activated by different agonists, including interleukin 1-beta, macrophage colony stimulating factor, or calcium ions. Most of the effects of C1P so far described seem to take place in intracellular compartments; however, the recent observation that C1P stimulates cell migration implicates a specific plasma membrane receptor that is coupled to a G protein. Therefore, C1P has a dual regulatory capacity acting as an intracellular second messenger to regulate cell survival, or as extracellular receptor ligand to stimulate chemotaxis.

Introduction

Normal development of an organism requires the intervention of complex biological processes that are strictly regulated to maintain cell and tissue homeostasis. These include systems to control cell growth and survival, as well as mechanisms to prevent disease. Alteration of any of these processes can lead to metabolic dysfunction or cause illnesses such as autoimmune diseases, chronic inflammation, neural degeneration, cardiovascular disorders, or cancer.¹⁻³

Many sphingolipids are crucial metabolites to control cell activation. Some of them have been described as key regulators of signal transduction processes that are essential for normal development. In particular, ceramides inhibit cell growth and are potent inducers of apoptosis, a form of programmed cell death.⁴⁻⁷ In neurons however, the situation is controversial as ceramides have been shown to promote either apoptosis or cell survival.⁸⁻¹² Also, ceramides play important roles in the regulation of cell differentiation, survival and inflammation⁹,¹³,¹⁵,²³,²⁸ and are key mediators of radiation and chemotherapy effects on tumors, bacterial and viral infections, heat or UVA injury and ischemia-reperfusion injury (Reviewed by Gulbins and Kolesnick). In addition, ceramides have been associated with insulin resistance through activation of protein phosphatase 2A and the subsequent dephosphorylation and inactivation of protein kinase B (PKB).³⁰⁻³² By contrast,

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Figure 1. Biosynthesis of sphingosine 1-phosphate and ceramide-1-phosphate.

Sphingosylphosphorylcholine, sphingosine-1-phosphate (S1P)\(^{33,34,37}\) and ceramide-1-phosphate (C1P)\(^{34,38-40}\) are potent stimulators of cell proliferation. As mentioned in previous chapters of this book, ceramides are generated by de novo synthesis, or can be produced by the action of different sphingomyelinases (SMases). Details on SMase activities, enzymology and compartmentalization are reviewed in several chapters included in this book. Natural ceramides typically have long N-acyl chains ranging from 16 to 26 carbons in length\(^7,43,44\) and some times longer in tissues such as skin. Many studies have used a short-chain analog (N-acetylsphingosine, or C\(_2\)-ceramide) in experiments with cells in culture because it is more water soluble than long-chain ceramides and it has been presumed that this compound did not occur in vivo. However, recent studies demonstrated that C\(_2\)-ceramide does exist in mammalian tissues. In particular, C\(_2\)-ceramide was found in rat liver cells\(^{45,46}\) and brain tissue.\(^{46}\)

Formation of ceramide is also relevant because it is the precursor of important bioactive sphingolipids that can also regulate cellular functions. For instance, stimulation of ceramidases results in generation of sphingosine (Fig. 1), which is a physiological inhibitor of protein kinase C (PKC).\(^{14}\) There are numerous reports showing that PKC is inhibited by exogenous sphingosine and Merril and coworkers demonstrated that addition of the ceramide synthase inhibitor fumonisin B1 to J774 macrophages to increase the levels of endogenous sphingoid bases, also inhibited protein kinase C.\(^{37}\) Sphingosine can control the activity of other key enzymes involved in the regulation of metabolic or cell signaling pathways such as the Mg\(^{2+}\) dependent form of phosphatidate phosphohydrolase,\(^{48,49}\) phospholipase D (PLD),\(^{50}\) or diacylglycerol kinase (DAGK)\(^{51,52}\) in different cell types. Sphingosine, in turn, can be phosphorylated by the action of sphingosine kinases to generate S1P, which is a potent mitogenic agent and can also inhibit apoptosis in many cell types.\(^{33,34,35,37,53,54}\) More recently, it was demonstrated that S1P stimulates cortisol\(^{55}\) and aldosterone secretion\(^{46}\) in cells of the zona fasciculata and zona glomerulosa, respectively, implicating S1P in the regulation of steroidogenesis.

A major metabolite of ceramide is ceramide-1-phosphate (C1P), which is generated through direct phosphorylation of ceramide by ceramide kinase (CerK) (Fig. 1). There is increasing evidence suggesting that C1P can regulate cell proliferation and apoptosis (Reviewed in refs. 24, 38) and the Chalfant laboratory group demonstrated that C1P is a key factor in inflammatory responses (Reviewed in refs. 57, 58). In addition, C1P plays a key role in phagocytosis (please see chapter by Hinkovska-Galcheva et al).\(^{59,60}\) The aim of the present chapter is to review recent progress related to the control of cell survival and the inflammatory response by C1P.

**Ceramide-1-Phosphate Synthesis and Degradation**

The only enzyme so far identified to produce C1P in mammalian cells is ceramide kinase (CerK). CerK was first observed in brain synaptic vesicles\(^{61}\) and later found in human leukemia HL-60 cells.\(^{11}\) This activity was first reported to be confined to the microsomal membrane fraction, but has also been reported to be mainly located in the citosol.\(^{52}\) These discrepancies might be due to