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Roles and regulation of neutral sphingomyelinase-2 in cellular and pathological processes

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A B S T R A C T

Our understanding of the functions of ceramide signaling has advanced tremendously over the past decade. In this review, we focus on the roles and regulation of neutral sphingomyelinase 2 (nSMase2), an enzyme that generates the bioactive lipid ceramide through the hydrolysis of the membrane lipid sphingomyelin. A large body of work has now implicated nSMase2 in a diverse set of cellular functions, physiological processes, and disease pathologies. We discuss different aspects of this enzyme's regulation from transcriptional, post-translational, and biochemical. Furthermore, we highlight nSMase2 involvement in cellular processes including inflammatory signaling, exosome generation, cell growth, and apoptosis, which in turn play important roles in pathologies such as cancer metastasis, Alzheimer's disease, and other organ systems disorders. Lastly, we examine avenues where targeted nSMase2-inhibition may be clinically beneficial in disease scenarios.

Introduction

Sphingomyelin hydrolysis is catalyzed by a class of enzymes referred to as sphingomyelinases (SMases) to generate ceramide, a bioactive lipid involved in diverse cellular processes (Hannun and

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Obeid, 2011; Ogretmen and Hannun, 2004). SMases are classified based on their pH optima of activity into acid, neutral, and alkaline subtypes. Of the four different mammalian neutral SMases that have been identified; neutral sphingomyelinase-2 (nSMase2) appears to be the predominant nSMase in cellular systems, physiologies, and pathologies (Airola and Hannun, 2013; Clarke et al., 2011b; Goni and Alonso, 2002; Wu et al., 2010). This review will focus on the roles and regulation of this enzyme emphasizing recent findings implicating nSMase2 in disease processes.

Characterization and regulation of nSMase2

Cloning

Cloning of nSMase2 revealed it as part of a protein superfamily that hydrolyzes phosphodiester linkages and requiring Mg^{2+} for activity. Both the human and mouse nSMase2 gene (SMPD3) encode for a 655 amino acid protein with a molecular mass of 71 kDa that contains an N-terminus with 2 hydrophobic segments and a C-terminus consisting of the catalytic site (Hofmann et al., 2000). The mouse and human versions are very similar and share 90% sequence identity.

Basic biochemical properties

NSMase2 specifically hydrolyzes the phosphocholine-headgroup from sphingomyelin and does not exhibit Phospholipase C-type activity against phosphatidylcholine, lyso-phosphatidylcholine, platelet activating factor or lyso-platelet activating factor. Neutral pH and divalent cations (Mg^{2+} or Mn^{2+}) are required for activity, while phosphatidylserine (PS) and unsaturated fatty acids stimulate enzymatic activity in vitro (Hofmann et al., 2000). A later study found that sphingosylphosphocholine, the deacylated form of SM, can be hydrolyzed under detergent-free conditions by nSMase2 (Miura et al., 2004).

Structural features

To date, there has not been a crystal structure reported for nSMase2. Therefore, our current understanding of the structure and mechanism of nSMase2 is based on investigations of related bacterial SMases. Three different structures of bacterial neutral SMases from the pathogenic organisms *Bacillus cereus*, *Listeria ivanovii*, and *Staphylococcus aureus* has confirmed neutral SMases belong to the DNase I type protein superfamily, which also includes inositol phosphatases (Ago et al., 2006; Huseby et al., 2007; Matsuo et al., 1996; Openshaw et al., 2005). NSMase2 shares relatively low sequence identity with the bacterial versions but most likely shares a similar protein fold and catalytic mechanism for SM hydrolysis (Ago et al., 2006; Openshaw et al., 2005). Studies by Tani et al. showed that the enzyme harbors two hydrophobic loops at the amino terminus rather than the sequence-predicted transmembrane segments (Tani and Hannun, 2007a).

Cellular localization

After initially cloning nSMase2, Hoffman et al. showed by antibody staining that nSMase2 localized to the Golgi apparatus in both PC12 and SH-SY5Y cells (Hofmann et al., 2000). Subsequently, overexpressed nSMase2 was shown to localize to the plasma membrane (PM) in the confluence phase of MCF7 cells and in primary hepatocytes (Karakashian et al., 2004; Marchesini et al., 2004). Building on these findings, Tani et al. found that nSMase2 localized to the inner leaflet of the PM in confluent MCF7 cells and was palmitoylated at two Cysteine clusters (Tani and Hannun, 2007a,b). The first cluster encompasses Cys53, Cys54 and Cys59 that are present between the 2 hydrophobic segments of the N-terminus, and the second cluster encompasses Cys395 and Cys396, which are present at the beginning of the catalytic site (Tani and Hannun, 2007b). Studies with cycloheximide in subconfluent MCF7 cells demonstrated that the Golgi localization of overexpressed subconfluent nSMase2 is the result of two protein pools: newly synthesized protein and a second that recycles back from the PM through the endosomal system (Milhas et al., 2010).