Neuromol Med (2010) 12:341–350
DOI 10.1007/s12017-010-8114-x

REVIEW PAPER

The Role of the Ceramide Acyl Chain Length in Neurodegeneration: Involvement of Ceramide Synthases

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Received: 24 December 2009 / Accepted: 29 April 2010 / Published online: 26 May 2010

Abstract Ceramide forms the backbone of all complex sphingolipids and has been the focus of considerable attention in the past few years due to the discovery that ceramide plays vital roles as an intracellular messenger. Ceramide, which consists of a sphingoid long chain base to which a fatty acid is *N*-acylated, is synthesized in mammals by a family of ceramide synthases (CerS), each of which uses a restricted subset of fatty acyl CoAs for *N*-acylation. Sphingolipids are found at high levels in nervous tissue, where they perform a variety of important functions in both the adult and the maturing brain. We now review what is known about the role of the acyl chain composition of ceramides and sphingolipids in normal brain development and in neurological diseases. Specifically, we attempt to integrate the information that is available about CerS expression and activity in the brain with the changes in the acyl chain composition of ceramide and complex sphingolipids in a number of neurodegenerative diseases and conditions, such as metachromatic leukodystrophy, neuronal ceroid lipofuscinoses, HIV infection, aging, Alzheimer's disease, ischemia, and epilepsy. We conclude that understanding the direct relationship between the CerS proteins and neurological conditions will be of great importance for delineating the precise roles of sphingolipids in the brain and is likely to be the subject of intense research activity in the years ahead.

Keywords Ceramide · Ceramide synthase · Sphingolipids · Acyl chain · Fatty acid · Neurodegeneration · Axons

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Abbreviations

ALS	Amyotrophic lateral sclerosis
ApoE	Apolipoprotein E
ASA	Arylsulfatase A
CerS	Ceramide synthase
CGT	Ceramide galactosyltransferase
CNS	Central nervous system
CSF	Cerebrospinal fluid
CST	Cerebroside sulfotransferase
GalCer	Galactosylceramide
GDF1	Growth/differentiation factor 1
GlcCer	Glucosylceramide
GSLs	Glycosphingolipids
HexCer	Hexosylceramide
HIVD	HIV dementia
Hox	Homeobox
MLD	Metachromatic leukodystrophy
NCL	Neuronal ceroid lipofuscinoses
PS1	Presenilin 1
SLs	Sphingolipids
SGalCer	Sulfogalactosylceramide
SM	Sphingomyelin
TLC	Tram-lag-CLN8
uog1	Upstream of GDF1

Introduction

All eukaryotic cells are surrounded by a membrane lipid bilayer, which consists of three major classes of lipids, namely glycerolipids, sphingolipids (SLs), and sterols. SLs are important structural components of membranes and are also involved in a broad range of regulatory functions (Lahiri and Futerman 2007). The precursor of all SLs is

ceramide, which consists of a fatty acid linked by an amide bond to the C-2 position of the sphingoid long-chain base, sphinganine or sphingosine. Ceramides are perhaps the best studied class of SLs because of their important roles as intracellular messengers regulating biological processes as diverse as cell growth, proliferation, differentiation, senescence, and apoptosis (Futerman and Hannun 2004).

Ceramide can be generated by de novo synthesis, by degradation of complex SLs such as sphingomyelin (SM) or glucosylceramide (GlcCer) or by re-acylation of sphingoid long-chain bases. In mammals, de novo ceramide synthesis is mediated by a family of six enzymes, the ceramide synthases (CerS) 1-6, each of which is the product of a unique gene. The six CerS preferentially use a relatively restricted subset of fatty acyl CoAs for *N*-acylation of the sphingoid long-chain base. Thus, CerS1 uses mainly C18-CoA; CerS4 uses C18- and C20-CoAs; CerS5 and CerS6 use mostly C16-CoA; and CerS3 uses very long acyl chain CoAs (C26 and higher; Pewzner-Jung et al. 2006). CerS2 can utilize a wider range of fatty acyl CoAs, but uses mainly C22 to C24 (Laviad et al. 2008). The expression pattern of the CerS, their structural features, localization, and regulation have been reviewed recently (Levy and Futerman 2010). Only a relatively small number of studies have addressed the biological role of the length of the fatty acid found in ceramide and SLs, and the discovery of the CerS should accelerate the process of determining the role of the acyl chain length in cell physiology.

The brain has the highest lipid concentration in the body apart from adipose tissue. Thus, dysregulated lipid metabolism may be of particular importance for central nervous system (CNS) injuries and disorders (Adibhatla and Hatcher 2008). SLs are highly enriched in the CNS, especially in myelin where SLs account for more than half of the total lipid content. However, although early research on SLs and on glycosphingolipids (GSLs), such as gangliosides, was stimulated by the detection of their high levels in nervous tissue (Schwarz and Futerman 1996; Yu and Saito 1989), little work has been performed on the role of ceramide and on ceramide containing specific acyl chain lengths, in the nervous system. This area is of particular importance since it is now becoming apparent that ceramide containing specific fatty acids play more vital roles in cell physiology than once thought, though what these roles are have not been fully delineated. It is known that ceramide with different acyl chain lengths has distinct biophysical properties (Pinto et al. 2008; Sot et al. 2005) and that the ceramides could themselves influence the biophysical properties of the membrane in which they are found by, for instance, differentially interacting with other membrane components. Evidence is also accumulating that ceramides with specific acyl chain lengths are generated in different signaling pathways and that these ceramides can differentially interact with downstream components in such pathways (Kroesen et al. 2003; Pewzner-Jung et al. 2006).

We now review what is currently known in this area and discuss the possible roles of CerS, and of the ceramide acyl chain length, in disorders of the CNS.

Lipid Composition of the CNS

The lipid composition of different brain areas and brain components was studied in detail in the 1960s and 1970s and has been extensively reviewed by others, who have documented, for instance, that different lipids are found in specific cell types and regions of the CNS (Baumann and Pham-Dinh 2001; O'Brien and Sampson 1965; Sastry 1985). The major source of CNS lipids is myelin, in which lipids constitute up to ~80% of the dry weight, significantly higher than white (50–66%) or gray matter (35–40%). All the major lipids found in whole brain are also present in myelin, and there are no lipid components that are exclusively present in myelin (O'Brien and Sampson 1965). However, cerebrosides (GlcCer and galactosylceramide (GalCer)) are the most typical lipids of myelin, representing 15–20% of the total lipids, with the cerebroside concentration in white matter ~10-fold higher than in gray matter. Most of the cerebroside in brain is GalCer, with the ceramide core enriched in very long chain fatty acids (C22–C24) (Baumann and Pham-Dinh 2001). During development, the GalCer concentration in the brain is proportional to the amount of myelin, as are the very long chain fatty acids.

SM accounts for ~5–13% of the brain phospholipid content. Higher concentrations of SM are found in peripheral nerves and in white matter, as SM is a major lipid of myelin. The SM of nervous tissue comprises mainly stearic (C18) and lignoceric (C24) acids. During the first 2 years of post-natal life, C18-SM decreases from ~80% of the total SM to ~30%, with a concomitant rise in C24-SM, from ~4 to ~33%; in other words, levels of medium long chain fatty acids in SM diminish and levels of very long chain fatty acids increase. The fatty acid composition of SLs in oligodendrocytes resembles that in myelin and is particularly rich in C24-fatty acids (Baumann and Pham-Dinh 2001).

Gangliosides occur at substantially higher levels in gray matter than in white matter, and this led to the erroneous conclusion that gangliosides are uniquely neuronal components; however, it is now known that gangliosides are found in all cell types of the CNS. The fatty acid composition of gangliosides comprises mainly C18-fatty acids (>80%) and remains unaltered from birth to old age in human brain (Sastry 1985). Astroglia contain the highest concentration of gangliosides, followed by neurons and

oligodendrocytes. Axons have ~14% lipid on a dry weight basis, which is much less than in any other cell type of the CNS. The fatty acids of axonal SLs are of shorter chain length than those from the corresponding myelin lipids and similar to that of gray matter, containing mainly C18-SLs.

The lipid content of gray matter and myelin does not vary significantly with age. However, the lipid content of white matter is lower in newborns (~50%) compared to young children (>60%). This change is due to the myelination process that takes place in the first years of life (O'Brien and Sampson 1965).

CerS Expression in the CNS

Since the discovery of the mammalian CerS in 2002 (Pewzner-Jung et al. 2006; Venkataraman et al. 2002), little progress has been made in determining the role of the CerS in the CNS. It is often assumed that CerS1 is the major CerS in the CNS, an assumption based on the limited expression pattern of CerS1, which is expressed mainly in the brain and in skeletal muscle (Laviad et al. 2008; Riebeling et al. 2003). By using in situ hybridization and RT–PCR (Becker et al. 2008), it was shown that CerS1 is expressed in the gray matter more than in the white matter and is expressed throughout the forebrain, in all layers of the neocortex, in pyramidal cells of the hippocampus, and in the dentate gyrus. High expression was also found in the brain stem and in the cerebellum (Becker et al. 2008). Together, this suggests that CerS1 is highly expressed in most neurons.

In contrast to the limited tissue distribution of CerS1, most other CerS have a much wider distribution, and analysis of CerS mRNA expression demonstrates that all other CerS, with the exception of CerS3, are also expressed in the CNS, albeit at lower levels than CerS1 (Fig. 1). Since the brain contains a variety of fatty acids in SLs and GSLs (see above), it is likely that CerS other than CerS1 (which synthesizes C18-ceramide, see below) are active in the CNS in the synthesis of ceramide. This is supported by analysis of the expression pattern of CerS4 and CerS6, which was similar to that of CerS1, although found at lower levels. CerS5 is expressed in nearly all brain regions, with comparable signal intensities in the gray and white matter suggesting similar expression levels in most cell types within the brain (Becker et al. 2008). CerS2 expression is high in all white matter tracts, including the corpus callosum, striatum, white matter of the cerebellum, and brain stem. This expression pattern is consistent with CerS2 expression in mature myelinating oligodendrocytes. CerS2 expression is also high in Schwann cells of the peripheral nervous system (Becker et al. 2008).

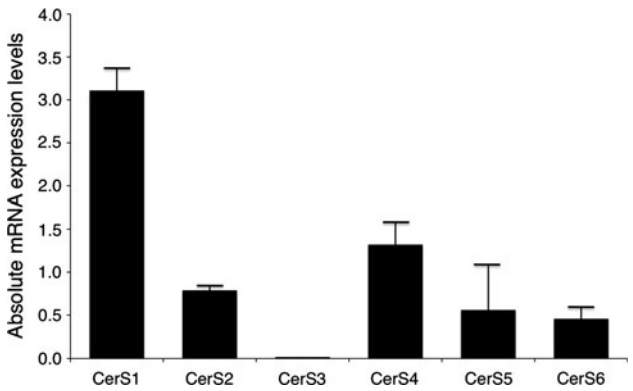


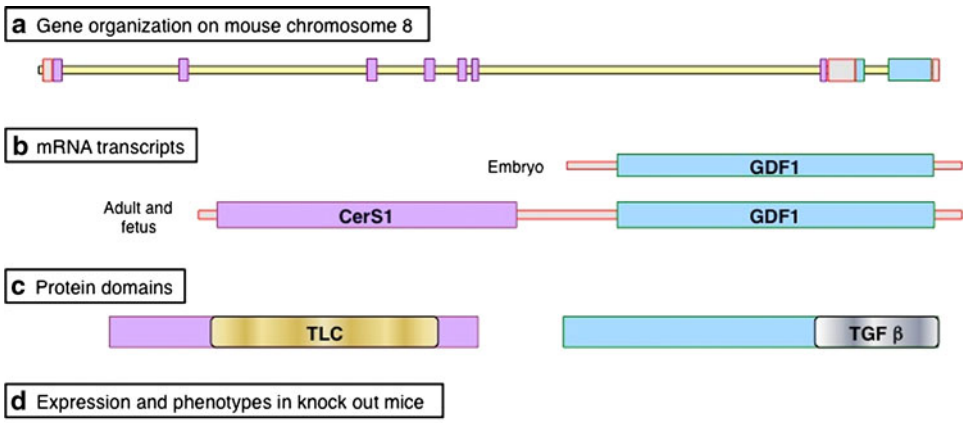
Fig. 1 CerS expression in the brain. CerS mRNA levels in 6–8-week-old mice as measured by quantitative RT–PCR. Adapted from (Laviad et al. 2008)

Little is known at present about the half-life of the CerS in biological tissues or about the half-life of individual ceramide or SLs containing different fatty acid species, rendering it very difficult to draw definitive conclusions about the role of each CerS and ceramide species in CNS physiology. It should also be noted that CerS1 turns over rapidly and is regulated by proteasomal mediated turnover in cells of CNS origin (glioblastoma cells) (Sridevi et al. 2009); much more work is needed before the significance of this finding can be extrapolated to understanding the role of CerS1 in the brain, but it nevertheless indicates a unique mode of post-translational regulation of CerS1 (Sridevi et al. 2010).

Does CerS1 Nevertheless Play a Unique Role in the Brain?

In addition to its restricted tissue distribution, CerS1 is somewhat distinctive in a number of other facets compared to CerS2-6. Thus, although CerS1 is homologous to the other mammalian CerS, it is on a separate branch of the phylogenetic tree (Pewzner-Jung et al. 2006) and is structurally, catalytically, and functionally unique. CerS1 is the only CerS which specifically catalyses the synthesis of C18-ceramide (Venkataraman et al. 2002), although CerS4 does show some activity toward C18-acyl CoA (Riebeling et al. 2003). Unlike CerS2-6 (Mesika et al. 2007), CerS1 does not contain a Hox-like domain; the Hox domain is derived from homeobox proteins, sequence-specific transcription factors important in development (Gehring et al. 1994). However, the first 15 amino acids of this domain are missing in CerS2-6, as is the key residue involved in DNA binding, rendering it unlikely that the Hox-like domain functions as a genuine transcription factor.

As mentioned above, CerS1 can be cleaved by proteasomal mediated turnover (Sridevi et al. 2009) concomitant with translocation to the Golgi apparatus (Sridevi et al.



d Expression and phenotypes in knock out mice

	CerS1	GDF1
Knock out mouse	?	Lethal
Conditional knock out mouse	?	No phenotype
Expression pattern in wild type	Brain and skeletal muscle	Brain, spinal cord and PNS

Fig. 2 Genomic organization, the bicistronic transcript, and protein domains of CerS1 and GDF1. **a** The genomic organization of CerS1 on mouse chromosome 8, upstream to GDF1; the open reading frame of CerS1 is shown in violet and the open reading frame of GDF1 in blue. **b** During embryogenesis, only a 1.4-kb mRNA fragment is detected, as opposed to the full length (3-kb) bicistronic mRNA found in the adult. **c** The mature CerS1 protein contains a TLC domain, and GDF1 contains a TGFβ domain. **d** The tissue distribution of CerS1 and GDF1, and the available information on the GDF1 knockout mouse

2010). In addition, CerS1 sensitizes non-neuronal cells to a wide range of drugs including cisplatin, carboplatin, doxorubicin, and vincristine (Min et al. 2007), CerS1 has been linked to head and neck squamous cell carcinoma (Senkal et al. 2007, 2009) and to sensitivity to chemotherapeutic drugs in cultured chronic myeloid leukemia cells (Baran et al. 2007).

Unexpectedly, CerS1 is synthesized as a bicistronic mRNA. CerS1 was originally discovered while screening for transforming growth factor β family members (Lee 1991) and was found at that time (prior to its discovery as a ceramide synthase) to be expressed as part of a bicistronic mRNA together with growth/differentiation factor-1 (GDF1) (Fig. 2); hence, its original name was *uog1* (upstream of growth/differentiation factor1). GDF1 is expressed in embryogenesis where it is necessary for right–left axis formation (Rankin et al. 2000). In the early embryonic stage, a 1.4-kb mRNA fragment that expresses only GDF1 is present. From embryonic day 9 and onwards, an additional 3-kb fragment is detected which consists of CerS1 and GDF1 in a bicistronic mRNA. In the adult mouse, only the 3-kb fragment is expressed; the 3-kb species is also found in adult spinal cord, cerebellum, and brain stem as well as in fetal brains from various developmental stages (Lee 1991). The bicistronic CerS1 mRNA was also found in two cultured cell lines from the nervous system (Lee 1991). This type of bicistronic organization is almost unprecedented among mammalian cellular mRNAs. The role of GDF1 in the adult mouse is not clear since a conditional deletion of GDF1 in the adult forebrain did not result in a phenotype even though efficient gene inactivation was achieved (Bengtsson et al. 2008). Interestingly, elevation of the level of the bicistronic mRNA was detected in the GDF1 conditional knockout mouse.

Whether the unique characteristics of CerS1 are important for its biological activity in the brain is not known. But it should be stressed that its unique expression pattern and its unique modes of regulation both transcriptionally and post-translationally, together with the high levels of C18-SLs and GSLs in the CNS, imply that CerS1 is likely to be a key player in the regulation of ceramides, SLs, and GSLs in the CNS.

CerS2 is Required for Myelination

CerS2 uses very long chain acyl CoAs (C22–C24) for ceramide synthesis (Laviad et al. 2008). CerS2 is the most ubiquitously expressed CerS and displays the broadest tissue distribution; the two tissues with the highest CerS2 mRNA levels, kidney and liver, have the highest proportions of C22–C24-ceramides. CerS2 expression is generally low in tissues expressing high levels of CerS1 or CerS3, such as brain, muscle, and skin, which corresponds to the somewhat lower levels of C22–C24-ceramides and C22–C24-SM. However, CerS2 is the predominant CerS in myelinating cells of the CNS and peripheral nervous system, which is in accordance with the enrichment of very long chain fatty acids in GalCer in white matter and in myelin (Becker et al. 2008). In brain and skeletal muscle, hexosylceramides (HexCer) contain surprisingly high proportions of C22–C24 acyl chains (Laviad et al. 2008).

A CerS2 knockout mouse was recently generated using a lacZ insertion, and analysis of this mouse sheds some light on the role of CerS2 in the nervous system (Imgrund et al. 2009). CerS2 is abundantly expressed in white matter tracts such as the corpus callosum and internal capsule, and in particular in oligodendrocytes. Widespread neuronal expression is also observed, but strikingly different levels of expression are observed in different neuronal populations. For instance, the LacZ reporter activity was abundantly detected in neocortical neurons of all layers, but its expression in the hippocampal formation was highly regionalized. Thus, CA1 pyramidal cells displayed the most intense lacZ staining anywhere in the CNS. In the cerebellum, lacZ staining was found both in white and gray matter, while Purkinje cells were not stained. CerS2 is not restricted uniquely to oligodendrocytes but is also found in specific neuronal populations.

The phenotype observed in the CerS2 knockout mouse suggests a strong association between CerS2 expression and myelin disorganization. Abnormalities in central and peripheral myelin sheaths in the inner lamella were found, which were focally detached in ~20% of all myelinated axons. Moreover, myelin basic protein was reduced by ~80% in CerS2 knockout mice, and myelin-associated glycoprotein was decreased by ~20% (Imgrund et al. 2009).

Ceramide and CerS in Neurodegeneration

The roles of ceramide and SLs in the CNS and their relevance to neuronal degeneration have been extensively reviewed (Adibhatla and Hatcher 2008; Arboleda et al. 2009; Buccoliero and Futerman 2003; Jana et al. 2009; Luberto et al. 2002; Morales et al. 2007). However, none of these reviews paid significant attention to the role of ceramide species containing different acyl chain lengths. Since the discovery of the six mammalian CerS, some new evidence has emerged that may provide a link between ceramide acyl chain length and neurodegeneration in various neurological disorders. This data is summarized in Fig. 3 and in Table 1 and is discussed in detail below.

Metachromatic Leukodystrophy

Metachromatic leukodystrophy (MLD) is a lysosomal storage disorder caused by the deficiency of arylsulfatase A (ASA). In the absence of this enzyme, sulfatides accumulate in many tissues, leading to destruction of the myelin sheath of the nervous system. In a mouse model of MLD, the CGT/ASA (−/−) mouse (in which ceramide galactosyltransferase (CGT) was overexpressed in an ASA (−/−) mouse), C18-sulfogalactosylceramide (SGalCer) accumulated to significant levels in neurons. These mice exhibited nerve fiber degeneration in the spinal cord, suggesting that C18-SGalCer accumulation in neurons may contribute to the disease phenotype (Eckhardt et al. 2007). In a different model of MLD, a CGT transgenic mouse (which overexpresses CGT in a normal ASA background), both C18-GalCer and C18-sulfatide accumulated in neurons. These mice have a reduced life span and display lethal audiogenic seizures after relatively mild acoustic stimulation. In a further mouse which overexpresses both CGT and cerebroside sulfotransferase (CST), even higher sulfatide levels were observed, although little GalCer accumulated. These mice were more sensitive to audiogenic seizures, implying

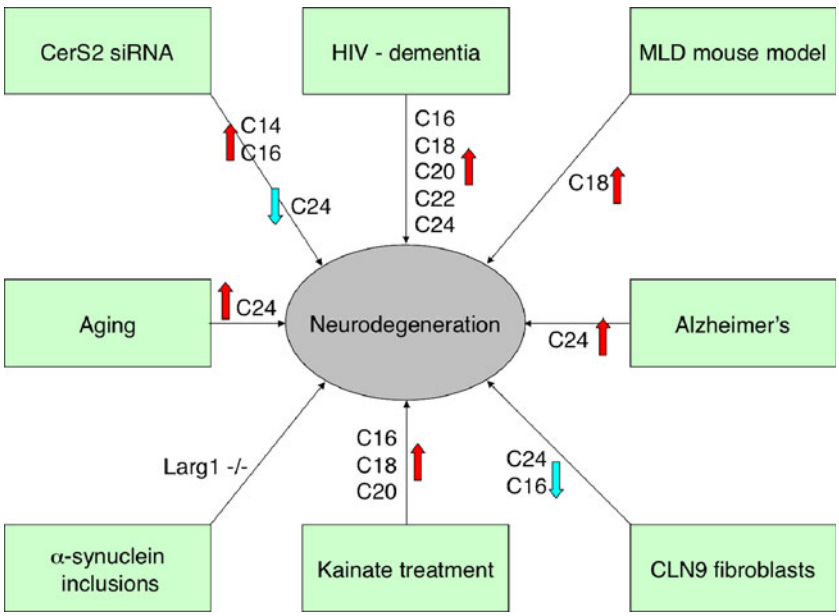


Fig. 3 Correlation between the acyl chain length of SLs and neurodegenerative disorders. Levels of SLs containing different acyl chain lengths are altered in a wide spectrum of neurological disorders, which may be of relevance for the underlying mechanism causing neuronal loss or degeneration

Table 1 Changes in the SL acyl chain length in neurological disorders

Disease or condition	Change in acyl chain length of SLs	Pathology	Reference
MLD mouse model (CGT/ASA −/−)	C18 SGalCer ↑	Neuronal death	(Eckhardt et al. 2007)
MLD mouse models (CGT and CGT/CST)	C18 GalCer and sulfatides ↑	Premature death of mice	(van Zyl et al. 2010)
CLN9 fibroblasts	C24 and C16 ceramide ↓	High apoptosis and high proliferation	(Schulz et al. 2006)
CLN9 + CerS1[a]	C18 ceramide ↑	Corrects the CLN9 phenotype	(Schulz et al. 2006)
HIV with dementia, brain tissue and CSF	C16, C18, C20 and C22 SM ↑ C16, C18 and C24 ceramide ↑	Neuronal dysfunction and death	(Haughey et al. 2004)
Cultured hippocampal neurons exposed to gp120 or Tat[b]	C16, C18, C20, C22 and C24 SM ↑ C16, C18 and C24 ceramide ↑	Neuronal death	(Haughey et al. 2004)
Alzheimer's patients (frontal gyrus)	C24 ceramide and GalCer ↑	Neuronal death	(Cutler et al. 2004a)
Alzheimer's mouse model (Presenilin 1)	C20 and C24 ceramide ↑	Astrocyte apoptosis after exposure to C20 ceramide	(Wang et al. 2008)
C. elegans[c]	Larg1 ↓	Elevation in α-synuclein inclusions	(van Ham et al. 2008)
Hippocampus of kainate-treated mice	C16, C18, and C20 ceramide ↑	Neuronal injury	(Guan et al. 2006)
Neuroblastoma + CerS2 siRNA[d]	C24 ceramide and SM ↓ C14 and C16 ceramide ↑	Induces autophagy	(Spassieva et al. 2009)

[a] CLN9 fibroblasts transfected with CerS1

[b] HIV retroviral proteins, gp120 (envelope protein) and Tat (transcriptional transactivator)

[c] A genetic screen for α-synuclein modifiers in *C. elegans*

[d] Neuroblastoma cells transfected with siRNA targeted to CerS2

that the seizures are related to the accumulation of C18-sulfatide (van Zyl et al. 2010), rather than C18-GalCer.

Neuronal Ceroid Lipofuscinoses

Neuronal ceroid lipofuscinoses (NCL) 9 is a genetic disorder in which neuronal death is caused by apoptosis. CLN9-deficient fibroblasts [CLN9 is the putative gene affected in NCL9 (Schulz et al. 2006)] have a distinctive phenotype of rapid growth and increased apoptosis, and ceramide levels and CerS activity are particularly low. Although neither mutations in the CerS genes nor changes in CerS expression were observed, CLN9-deficient fibroblast displayed a significant decrease in C16- and C24-ceramide. Interestingly, transfection with CerS1 caused elevation in C18-ceramide levels and partially corrected the phenotype of the CLN9 fibroblasts, and transfection with other CerS also corrected the phenotype (with the exception of CerS3). Based on these findings, it was suggested that CLN9 deficiency is in some way related to the regulation of CerS (Schulz et al. 2006), although an alternative explanation is that the ability of CerS to revert the CLN9 phenotype is not directly related, but rather a secondary or downstream effect.

Interestingly, CLN8 shows significant sequence similarity to the CerS (Pewzner-Jung et al. 2006), although no direct connection between any of the Batten's disease subtypes and CerS proteins has been demonstrated. Mutations in CLN8 (Vantaggiato et al. 2009) are associated with two distinct phenotypes: progressive epilepsy and mental retardation, and late infantile NCL. The role of the CLN8 protein is not known, and no connection has been shown between CLN8 and sphingolipids.

HIV Infection

Infection with HIV often causes neurological dysfunction including HIV-induced dementia (HIVD). In the cerebellum, the concentrations of C18- and C20-SM were significantly greater in samples from patients with mild and severe HIVD compared to controls and to patients that did not display dementia. In the cerebrospinal fluid (CSF), the concentrations of C16-, C18-, C20-, and C22-SM were significantly higher in severe patients compared with

controls (Haughey et al. 2004), and in different brain areas (medial frontal gyrus, parietal cortex, and cerebellum) of patients with HIVD, C16-, C18-, and C24-ceramide and GalCer were elevated. Interestingly, similar changes in the SL profile were observed when cultured cells were exposed to the HIV retroviral-specific proteins, gp120 (an HIV envelope protein) and Tat (an HIV transcriptional transactivator). Thus, hippocampal neurons exposed to gp120 exhibited elevation in C16-, C18-, C20-, C22-, and C24-SM 6 h after exposure, and elevation in C16-, C18-, and C24-ceramide after 12 h (Haughey et al. 2004).

Similar elevations in ceramide and SM were found in patients with HIVD with an apolipoprotein (ApoE) 4 genotype compared to patients with HIVD with an ApoE3 genotype. The ApoE4 genotype was found to be associated with faster rate of cognitive decline in Alzheimer's disease, with a worse prognosis after stroke and with a higher rate of neurologic complications in patients with HIV. It could be that an ApoE4 genetic background sensitizes neurons to dysfunctions in lipid metabolism (Cutler et al. 2004a).

Several cohort studies have now been performed to examine the involvement of SLs in HIV infection. The combined findings from these studies suggest that SM and ceramides with fatty acid chain lengths of C16 and C24 are dysregulated in the brains of patients with HIV-associated neurological disorders (Haughey et al. 2008). To date, no studies have examined the expression levels of the CerS proteins in HIV infection, so it is currently not possible to distinguish between the potential roles of changes in CerS expression or activity versus changes in the activity of other enzymes that metabolize SLs.

Alzheimer's Disease

A number of studies have suggested a causal link between ceramide or SL levels and Alzheimer's disease. For instance, significant increases were observed in C24-ceramide and C24-GalCer levels in the middle frontal gyrus (an area affected in Alzheimer's disease), but not in the cerebellum of patients with Alzheimer's (Cutler et al. 2004b). Upon comparing severe, moderate, and mild forms of Alzheimer's, levels of C18- and C24-ceramides were also increased, with the extent of the increase positively correlated with disease severity, whereas GalCer and sulfatide levels were not altered. When cultured hippocampal neurons were exposed to β-amyloid, levels of C18- and C24-ceramides, cholesterol and cholesterol esters were increased within 6 h of exposure, a time-point before neuronal death, as were levels of C24-SM. Based on these findings, a critical and essential role for perturbed SL metabolism and ceramide production in the neurotoxic actions of β-amyloid was proposed (Cutler et al. 2004b).

Presenilin 1 (PS1) knockout mice are a model of early familial Alzheimer's disease (Gautheron et al. 2009). Quantitative analyses demonstrated that PS1 tissue contains $\sim$3-fold more total ceramide than wild-type tissue. In particular, the proportion of C20- and C24-ceramide was increased by 4- and 8.5-fold, respectively, while the proportions of C16- and C18-ceramide remain unchanged (Wang et al. 2008). The predominance of C20- and C24-ceramide is concurrent with the elevated expression of CerS2 and CerS4. C20-ceramide induced apoptosis in hippocampal astrocytes from PS1 brains, but had a negligible effect in astrocytes from wild-type brain. Moreover, neuronal cultures exposed to C20-ceramide did not show as dramatic an effect since neurons did not undergo apoptosis.

Recently, a genome-wide RNA interference study on *C. elegans* was performed to identify genes involved in the formation of synuclein inclusions, and 80 genes were identified that, when knocked down, resulted in a premature increase in the number of inclusions (van Ham et al. 2008). One of the genes was Larg1, a *C. elegans* homolog of mammalian CerS1. Deletion of Lagr1 resulted in an increase of $\sim$35% in the number of α-synuclein inclusions in 9-day-old adult worms.

Together, these findings support the notion that there is a link between Alzheimer's disease and ceramide species, though the possibility cannot be excluded that some of the changes observed reflect a general change in SL metabolism rather than specific changes in the CerS proteins. Distinguishing between these two possibilities requires study of changes in the expression levels of all of the enzymes found in the pathways of SL synthesis and degradation and correlation of any changes with the changes in the SL profile.

Ischemia and Epilepsy

Kainate is a glutamate analog that has been widely used in pharmacological studies of neuronal injury related to ischemic conditions and epilepsy. A progressive elevation in levels of C16-, C18-, and C20-ceramide was observed in hippocampal neurons isolated from mice injected with kainate (Guan et al. 2006), although it is unclear whether changes also occurred in shorter and longer acyl chain ceramides and in SLs. Using a mouse monoclonal anti-ceramide antibody, the control hippocampus showed very light or no immunolabeling for ceramide, but kainate-injected animals showed loss of neurons in the CA1 and CA3 regions of the hippocampus and increased immunoreactivity to ceramide. Notably, at least at early stages, increased ceramide-labeling was largely restricted to neurons rather than glia cells in the hippocampus. Thus, accumulation of ceramide may be restricted primarily to

neurons at the early stage of neuronal degeneration, at least in kainate-treated mice.

Aging

A correlation between C24-SLs and aging has been reported (Cutler et al. 2004b). Concentrations of C24-ceramide in the cerebral cortex increased in an age-dependent manner such that levels in 25-month-old mice were 3-fold greater than in 3-month-old mice. Moreover, there was a dramatic (100-fold) age-related increase in C24-GalCer levels. Whether this correlates with changes in CerS expression during aging is not yet known.

Cell Culture Studies

In cultured hippocampal neurons, SLs have been shown to play three distinct roles in regulating neuronal development, with ceramide involved in initial stages of neuronal development and in apoptotic pathways (Brann et al. 1999, 2002; Schwarz and Futerman 1997), while GlcCer is involved in specific events of axonal growth and branching (Boldin and Futerman 1997, 2000; Mitoma et al. 1998). Thus, ceramide enhances the formation of minor processes from lamellipodia, and GlcCer synthesis is required for normal axon growth. Most importantly, at both of these stages, ceramide can induce apoptotic cell death at high concentrations. Neither exogenously added ceramide nor neutral sphingomyelinase affected cell viability at low concentrations, but at higher concentrations, both induced cell death via apoptosis at early and later stages of development.

Exposing neuroblastoma cells to CerS2 siRNA caused a decrease in C24-ceramide and -SM and an increase in C14- and C16-ceramide (Spassieva et al. 2009). Expression levels of CerS5 and CerS6 were also elevated. Neuroblastoma cells in which CerS2 was down regulated by siRNA did not exhibit apoptosis, but rather CerS2 down-regulation appeared to induce autophagy in these cells.

Others

Some studies of multiple sclerosis have shown that the neutral sphingomyelinase–ceramide pathway is involved in mediating oxidative stress-induced apoptosis and cell death in human primary oligodendrocytes (Jana et al. 2009). Also, abnormal buildup of SM, ceramide, and cholesterol esters has been observed in amyotrophic lateral sclerosis (ALS), and in mouse models of ALS, this abnormal lipid accumulation occurs in transgenic mice prior to any sign of cell death, but no specific chain lengths of SLs have been examined (Jana et al. 2009).

Concluding Remarks

The discovery of the six CerS has stimulated renewed focus on the role of the acyl chain length of ceramide and SLs with significant information becoming available recently, at least in non-neuronal tissues, implying that the acyl chain length is of great importance in both physiology and in pathophysiology [see, for instance, (Erez-Roman et al. 2010; Mizutani et al. 2009)]. However, much less is known at present about the precise roles of the CerS in the CNS, although levels of ceramides and SLs with defined acyl chains lengths do change in a number of neurological conditions, as discussed above. However, in a number of these conditions, no obvious acyl chain specificity is apparent, suggesting general changes in SL metabolism rather than specific changes in CerS, whereas in others, specificity does exist with respect to acyl chain length. It is the latter case in which the CerS are likely to be directly involved. While there are exciting clues that CerS play key roles in the nervous system, and in neurological diseases, determining the full extent of their involvement will progress more rapidly once the precise distribution and expression pattern of all the CerS is mapped in detail in the developing and adult brain, and once the mechanisms of regulation of CerS activity are determined, together with delineation of the relationship between levels of CerS expression and activity and levels of ceramide and SL species. This will presumably be achieved once more CerS knockout mice become available and once more appropriate tools (such as development of better antibodies to endogenous CerS, along with mass spectrometry imaging for SLs in the brain) become more established.

Acknowledgments Work in the Futerman laboratory on the CerS proteins is supported by the Israel Science Foundation (1404/07), the National Institutes of Health (GM076217), the Minerva Foundation, and the U.S.-Israel Binational Science Foundation. A.H. Futerman is the The Joseph Meyerhoff Professor of Biochemistry at the Weizmann Institute of Science and the head of the Nella and Leon Benoziyo Center for Neurological Diseases.

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