Necessary Role for the Lag1p Motif in (Dihydro)ceramide Synthase Activity*

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**Lag1** (longevity assurance gene 1) homologues, a family of transmembrane proteins found in all eukaryotes, have been shown to be necessary for (dihydro)ceramide synthesis. All Lag1 homologues contain a highly conserved stretch of 52 amino acids known as the Lag1p motif. However, the functional significance of the conserved Lag1p motif for (dihydro)ceramide synthesis is currently unknown. In this work, we have investigated the function of the motif by introducing eight point mutations in the Lag1p motif of the mouse LASS1 (longevity assurance homologue 1 of yeast Lag1). The (dihydro)ceramide synthase activity of the mutants was tested using microsomes in HeLa cells and in vitro. Six of the mutations resulted in loss of activity in cells and in vitro. In addition, our results showed that C18:0 fatty acid CoA (but not cis-C18:1 fatty acid CoAs) are substrates for LASS1 and that LASS1 in HeLa cells is sensitive to fumonisin B1, an in vitro inhibitor of (dihydro)ceramide synthase. Moreover, we mutated the Lag1p motif of another Lag homologue, human LASS5. The amino acid substitutions in the human LASS5 were the same as in mouse LASS1, and had the same effect on the in vitro activity of LASS5, suggesting the Lag1p motif appears to be essential for the enzyme activity of all Lag1 homologues.

In recent years, the sphingolipid ceramide has become established as a bioactive lipid regulating cellular senescence, apoptosis, and stress responses (1). It is, therefore, important to understand how enzymes that generate ceramide function and how they are regulated. The focus of this study is to understand how enzymes that generate ceramide function and in mitochondria-rich fractions before the gene or genes encoding this enzyme were known (3–5). Interestingly, the first (dihydro)ceramide synthase genes were not initially cloned as genes involved in ceramide synthesis but as **Uogi** (upstream of growth and differentiation factor 1) in mammals (6), **Lag1** (longevity assurance gene 1) in yeast (7), and **Ascl** (Alternaria stem cancer resistance gene 1) in tomato (8). New homologues of the yeast **Lag1** have been described in previous studies (9–14), and the products of some of the homologues were found to be involved in ceramide synthesis (15–18). Currently, this large family of membrane proteins comprises homologues in all genetically studied eukaryotes and some prokaryotes such that there are six paralogues in mice, six in humans, three in Arabidopsis, and two in baker’s yeast (11). According to recently accepted nomenclature, the six paralogues in mammals are referred to as LASS (longevity assurance homologues) (9). Different LASS paralogues show different specificity for the chain length of the fatty acid CoA substrate; e.g. LASS1/UOG1 utilize C18 CoA (17), and LASS5 prefers C16 CoA (13, 19).

Two recent publications provide evidence suggesting (dihydro)ceramide synthase might operate differently in different organisms. In yeast, there is an additional subunit necessary for the reaction, Lip1p (Lag1/Lac1 interaction protein) (20). Lahiri and Futerman (14) provide evidence in mammals for the LASS5 homologue as a bona fide (dihydro)ceramide synthase.

All members of the LASS/Lag family of proteins share similar transmembrane profiles of four to seven predicted transmembrane domains (11). Membrane localization of some of the members, including yeast Lac1p (longevity assurance gene cognate 1) (21) and mammalian LASS1, LASS4, LASS5, and LASS6 (13, 17, 19), is established to be in the endoplasmic reticulum. The LASS/Lag proteins share similar transmembrane profiles with a larger group of proteins, such as translocating chain-associating membrane (TRAM)2 protein, and a protein mutated in neuronal ceroid lipofuscinoses, CLN8 (22). This
TABLE 1
Primers used in site-directed mutagenesis experiments for generating Lag1p motif mutants of mouse LASS1

| Mutation | Primers                  |
|----------|--------------------------|
| H182D F  | 5′-GTGTCGAGTAGTCCTGTCG-3′ |
| H183D F  | 5′-TCGTCTGAACTGACCTGTC-3′ |
| L189E F  | 5′-GAGGCGGATCTTCCTGTCCT-3′ |
| L190E F  | 5′-AGAGCCTGCAGGACTCCTGTC-3′ |
| S193A F  | 5′-GTCCGCAGCTGCGGGGACTGTC-3′ |
| D210N F  | 5′-GTCGAGGCACTGAGGAGTTC-3′ |
| D213N F  | 5′-CTGAGACTGACCGGGAGTTC-3′ |
| L216E F  | 5′-GTCGAGGGAACGAGTTC-3′ |
| K220L F  | 5′-GCGGCGGCTGTCGTCG-3′ |
| R        | 5′-GCCGAGGCTGTCGTCG-3′ |

The underlined nucleotides depict mutated codons. F, forward; R, reverse.

TABLE 2
Primers used in site-directed mutagenesis experiments for generating Lag1p motif mutants of human LASS5

| Mutation | Primers                  |
|----------|--------------------------|
| H220D F  | 5′-ACTCTCTTPGAATGGGATGAC-3′ |
| S231A F  | 5′-CCATGGCTTCTCCTGACATGAA-3′ |
| L254E F  | 5′-GGACAGAACCTGACTGCTGAC-3′ |
| L254M F  | 5′-GGACAGAACCTGACTGCTGAC-3′ |

The underlined nucleotides depict mutated codons. F, forward; R, reverse.

not shown). For LASS5, a 2-kb NotI fragment containing human LASS5 was taken from pCMV6 vector (OriGene, Albert Park, Australia) and subcloned into the NotI site of the pCMV-EXSVneo vector. The direction of the insertion was verified. The 0.3-kb 5′ end of the human LASS5 open reading frame was amplified by PCR to introduce a 5′ end FLAG tag (forward primer, 5′-GCC CTC GAG ATG ATG GAT TAC AAG GAT GAC-3′; reverse primer, 5′-CCT CCA GCC TTT TCT TAT CAG CAG-3′). The 5′ FLAG-tagged PCR fragment of LASS5 was digested with XhoI and HindIII and used to replace the 5′ end of wild-type LASS5. Point mutations in the Lag1p motif of LASS5 were introduced by site-directed mutagenesis, as described for LASS1 (see above), with primers shown in Table 2.

**Transfection of HeLa Cells and Preparation of Microsomes**—HeLa cells were transfected with wild-type LASS1 or LASS5, Lag1p motif mutants, and empty vector constructs using Effectene™ reagent (Qiagen, Valencia, CA) according to the manufacturer’s instructions. Cells were harvested 48 h after transfection and lysed in 20 mM HEPES buffer (pH 7.4, 2 mM KCl, 2 mM MgCl₂, 250 mM sucrose) with an insulin syringe. Lysates were centrifuged at 1000 × g at 4 °C for 5 min to remove unlysed cells and nuclei. The supernatants were then centrifuged at 8000 × g at 4 °C for 10 min to remove mitochondria. Thereafter, the supernatants were ultracentrifuged at 100,000 × g at 4 °C for 1 h to collect microsomes. Microsomes were resuspended in HEPES buffer (see above), and protein concentrations were measured using the Bradford method (Bio-Rad).

**Western Blot Analysis**—Protein samples were separated on a 12% SDS gel (Bio-Rad) and transferred to a nitrocellulose membrane (Amersham Biosciences) using standard techniques (24). FLAG-tagged proteins were labeled with M2 FLAG (Sigma) as a primary antibody and goat anti-mouse-horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA) as a secondary antibody and detected with the ECL detection system (Amer- sham Biosciences).

**In Vitro Ceramide Synthesis**—Ten μg of microsomes were used as an enzyme source for the *in vitro* ceramide synthesis assay. A 100-μl reaction mix containing 15 μM C17 dihydrophosphinosine (DHS) (Avanti Polar Lipids, Alabaster, AL) and 50 μM C18, C18:1, or C16 fatty acid CoA (Avanti Polar Lipids) in 25 mM potassium phosphate buffer, pH 7.4, was prewarmed at 37 °C for 5 min. The enzyme reaction was started by adding microsomes to the reaction mix, the reaction mix was incubated at 37 °C for 15 min, and then the reaction was stopped by
Lag1p Motif

adding 2 ml of extraction solvent, ethyl acetate/2-propanol/water (60/30/10 v/v), supplemented with an internal standard for mass spectrometry analyses (see below). Lipids were extracted two times using 2 ml of extraction solvent, dried under a stream of nitrogen, and then resuspended into 150 μl of 1 mM NH₄COOH in 0.2% HCOOH in methanol and analyzed by mass spectrometry.

Phosphate Measurements—The phosphate content of the lipid extracts was measured with a standard curve analysis and colorimetric assay of ashed phosphate (25) and was used to normalize the mass spectrometry measurements of the lipids.

Mass Spectrometry Analyses—Electrospray ionization/tandem mass spectrometry analyses of endogenous and C17 sphingosine backbone ceramide species were performed on a Thermo Finnigan TSQ 7000 triple quadrupole mass spectrometer operating in a multiple reaction-monitoring positive ionization mode using a modified version of the published protocol (30). Briefly, cell pellets corresponding to ∼2–3 × 10⁶ cells were fortified with the internal standard, N-palmitoyl-d-erythro-C₁₃ sphingosine, and extracted with an ethyl acetate/isopropyl alcohol/water (60/30/10 v/v) solvent system. After evaporation and reconstitution in 150 μl of 1 mM NH₄COOH in 0.2% HCOOH in methanol, the samples were injected on the HP1100/TSQ 7000 liquid chromatography/mass spectrometry system and gradient-eluted from the BDS Hypersil C8, 150 × 3.2 mm, 3-μm particle size column with a 1.0 mM methanolic ammonium formate/2 mM aqueous ammonium formate mobile phase system. Peaks corresponding to the target analytes and internal standards were collected and processed using the Xcalibur software system. Quantitative analysis was based on the calibration curves generated by spiking an artificial matrix with the known amounts of the target analyte synthetic standards and an equal amount of the internal standard. The target analyte/internal standard peak area ratios were plotted against the analyte concentration. The target analyte/internal standard peak area ratios from the samples were similarly normalized to the internal standard and compared with the calibration curves using a linear regression model.

RESULTS

Mutations in the Lag1p Motif of LASS1 Affect Its Function in HeLa Cells—To investigate the functional significance of the Lag1p motif, we chose eight conserved amino acids within the motif and created eight individual point mutants in an N-terminal FLAG-tagged mouse LASS1 as depicted in Fig. 1B. The substitutions were designed to significantly alter the properties of the amino acid residues. It has already been shown that overexpression of LASS1 results in an increase of C18 ceramide (17). Therefore, the Lag1p motif mutants were overexpressed in HeLa cells and tested for their ability to increase cellular C18 ceramide. Forty-eight hours after transfection, the cells were harvested and subjected to lipid analysis by mass spectrometry. Fig. 2, A and B, show that, as compared with vector-control overexpressing cells, overexpression of wild-type LASS1 increased cellular levels of C18 and C18:1 ceramides by 10-fold. Interestingly, six of eight Lag1p motif mutants, when overexpressed in HeLa cells, showed no significant increase in cellular levels of C18 and C18:1 ceramides, suggesting that these mutations impaired the function of LASS1. However, the two mutants S193A and K220L were able to increase cellular C18 ceramides. Therefore, the Lag1p motif mutants were overexpressed in HeLa cells and tested for their ability to increase cellular C18 ceramide. Forty-eight hours after transfection, the cells were harvested and subjected to lipid analysis by mass spectrometry.
Lag1p Motif

FIGURE 2. Mass spectrometry measurements of ceramide species in HeLa cells overexpressing wild-type LASS1 or Lag1p motif mutants. Shown are C18 (A); C18:1 (B); and C16, C24, and C24:1 ceramides (C). In the sample transfected with the empty vector, the amount of the ceramides are C16, 0.34 pmol/nmol phosphate; C24, 2.16 pmol/nmol phosphate; and C24:1, 2.66 pmol/nmol phosphate. D: Western blot results with anti FLAG antibody. HeLa cells were transiently transfected with the wild-type LASS1, individual Lag1p motif mutants (all with an N-terminal FLAG epitope), or vector control, and ceramides were measured 48 h after transfection. Results in this figure are representative of three independent experiments.

and then used those microsomes as an enzyme source in an in vitro assay. The in vitro (dihydro)ceramide synthase assay was performed using a non-radioactive and highly sensitive mass spectroscopy-based method. This method utilizes C17 DHS, a non-naturally occurring sphingoid base, as a substrate for the (dihydro)ceramide synthase reaction, which is then converted into a non-naturally occurring C17 dihydroceramide. Both molecules can be distinguished from naturally occurring sphingoid bases and ceramides by mass spectrometry, thus allowing accurate measurement of in vitro ceramide synthase activity (for details, see “Experimental Procedures”). Using this assay with C17 DHS (15 μM) as a long-chain base substrate and C18 fatty acid CoAs (50 μM) as a fatty acid CoA substrate, it was seen (Fig. 3A) that microsomes prepared from cells overexpressing wild-type LASS1 and the mutants S193A and K220L, when compared with control microsomes, were the only ones exhibiting an increase of in vitro (dihydro)ceramide synthase activity. The remaining six mutants that did not show ceramide increases in cells also did not show a significant increase in activity as compared with the control. Therefore, these in vitro results with C18 fatty acid CoA as a substrate are in agreement with the data obtained from the whole cell experiments in Fig. 2A and further support the hypothesis that the Lag1p motif is important for the (dihydro)ceramide synthase activity of LASS1.

C18:1 (cis) Fatty Acid CoAs Are Not in Vitro Substrates for LASS1—Because LASS1 overexpression caused an increase in cellular levels of C18:1 ceramide as well as C18 ceramide, the in vitro (dihydro)ceramide synthase activity assay was also performed using oleoyl-CoA (C18:1 cis-Δ9). Fig. 3B demonstrates that microsomes obtained from cells overexpressing wild-type LASS1, when compared with microsomes from vector control-transfected cells, showed no significant increase of in vitro oleoyl-CoA ceramide synthase activity. Moreover, the mutants S193A and K220L also failed to show an increase of in vitro (dihydro)ceramide synthase activity with oleoyl-CoA as a substrate (data not shown). Because the mass spectroscopy method used to measure ceramide species in cells does not discriminate between C18:1 positional isomers, an additional C18:1 positional isomer, namely vaccenoyl CoA (C18:1 cis-Δ11), was tested. Fig. 3C demonstrates that, when vaccenoyl CoA was used as a substrate for the in vitro ceramide synthase activity assay, microsomes from cells overexpressing LASS1 also failed to show an increase of activity as compared with the control. These data indicate that LASS1 does not utilize cis-C18:1 fatty acid CoAs as substrates in vitro.

LASS1 Is a Fumonisin B₁-sensitive (Dihydro)ceramide Synthase—Fumonisin B₁ is a known inhibitor of (dihydro)ceramide synthase in vitro (26). However, in human embryonic kidney 293T cells overexpressing LASS1, fumonisin B₁ treatment was shown to increase cellular levels of C18 ceramide, suggesting that LASS1 is fumonisin B₁-insensitive (17). Therefore, it was interesting to test whether mutations in the Lag1p motif would alter the fumonisin B₁ effect on LASS1 in cells. Wild-type LASS1, the eight mutants, and the vector control constructs were transiently transfected in HeLa cells. Twenty-four hours post-transfection, the cells were treated for an additional 24 h with 20 μM fumonisin B₁. Mass spectrometry measurements of ceramide in fumonisin B₁-treated and -untreated cells are shown in Fig. 4. Interestingly, it was found that fumonisin B₁ treatment did not increase the levels of C18/C18:1 ceramides in cells overexpressing wild-type LASS1, but, in fact, significantly decreased C18/C18:1 ceramide levels when compared with untreated samples. This indicates that LASS1 is sensitive to fumonisin B₁ when overexpressed in HeLa cells. Furthermore, fumonisin B₁ treatment significantly decreased the levels of C16 ceramide (Fig. 4C) and, indeed, the levels of all ceramide species measured (data not shown). As can be seen in Fig. 4, fumonisin B₁ had similar effects on ceramide levels for all of the Lag1p motif mutants, indicating that mutation of the Lag1p motif did not affect the fumonisin B₁ sensitivity of LASS1.

The Lag1p Motif Is Necessary for (Dihydro)ceramide Synthase Activity of LASS Proteins—The eight amino acids mutated in the Lag1p motif of LASS1 are conserved in all of the LASS
proteins, but different LASS proteins have different fatty acid CoA specificity (13), and therefore, it is possible that they do not require the Lag1p motif for their activity. To address whether the Lag1p motif is required for the ceramide synthase activity of other LASS family proteins, the Lag1p motif of a different LASS homologue was investigated. To this end, four point mutations were introduced in the Lag1p motif of the human LASS5 cDNA. Three of the mutations, H220D, S231A, and L254E, were the same substitutions as in the Lag1p motif of LASS1 (Fig. 1, B and C) to directly compare the effect of these mutations on the activity of LASS1 and LASS5. The fourth point mutation L254M is at the same amino acid position as L254E but is a more conservative substitution. Of note is that methionine is present at this amino acid position of the Lag1p motif of another LASS/Lag homologue, namely the tomato ASC1 (Fig. 1A).

HeLa cells were transiently transfected with wild-type LASS5, LASS5 Lag1p motif mutants, and empty vector. Forty-eight hours after transfection, the cells were collected and used for the preparation of microsomes. Subsequently, the microsomes were used as an enzyme source in an in vitro (dihydro)ceramide synthase assay with C17 DHS (15/9262 M) as the long-chain base substrate and C16 fatty acid CoA (50/9262 M) as the fatty acid CoA substrate. The reactions were performed at the same conditions as the ones described for LASS1 (see above and “Experimental Procedures”). Fig. 5 demonstrates that overexpression of the wild-type LASS5 significantly increased (~20-fold) the in vitro (dihydro)ceramide synthase activity with C16 fatty acid CoA as a substrate. Moreover, the H220D and L254E amino acid substitutions severely impaired the in vitro (dihydro)ceramide synthase activity of human LASS5, whereas...
the S231A and L254M mutations did not significantly impact the ceramide synthase activity of human LASS5. The H220D, S231A, and L254E mutations had the same effect on the in vitro activity of human LASS5 as the H182D, S193A, and L216E mutations had on the in vitro activity of mouse LASS1 (Figs. 3A and 5), indicating that the Lag1p motif is necessary for the (dihydro)ceramide synthase activity of both LASS homologues and suggesting that the Lag1p motif is not only a conserved structural feature of LASS/Lag proteins but is required for their activity. The fact that the L254M mutant showed similar in vitro activity as the wild-type LASS5, whereas the L254E mutant was inactive, suggests that only conserved mutations are tolerated at this position of the Lag1p motif and additionally supports the importance of the motif for ceramide synthase activity.

In addition, we investigated the effect of the overexpression of the wild-type LASS5 and Lag1p motif mutants on cellular ceramide levels in the presence and absence of fumonisin B1. HeLa cells were transiently transfected with wild-type LASS5, the Lag1p motif point mutants, or an empty vector. Half of the samples were treated with 20 μM fumonisin B1. The fumonisin B1 treatment started 24 h after the transfection and was performed for another 24 h. All of the samples were collected 48 h after the transfection and subjected to mass spectrometry analyses. Surprisingly, and in contrast to the effect of LASS5 overexpression on the in vitro activity of microsomes (Fig. 5), the overexpression of LASS5 did not significantly increase the levels of C16 ceramide in cells (Fig. 6A). The same was true for the S231A and L254M mutants, which exhibited an increase in their in vitro activity. In addition, C14 ceramide levels (Fig. 6B) were also not significantly affected by the overexpression of the wild-type LASS5 or the Lag1p motif mutations. C14 fatty acid CoA was previously shown to be one of the preferred substrates for LASS5 in vitro (13). C24 ceramides (Fig. 6C) and the other measured ceramide species (data not shown) were not changed by the overexpression of wild-type LASS5 or the Lag1p motif point mutations. Taken together, the measurements of ceramide levels in cells and the in vitro (dihydro)ceramide synthase activity of samples overexpressing the wild-type LASS5 or the Lag1p motif mutants suggest that, in HeLa cells, de novo synthesis of C16 ceramide is likely to be tightly regulated.

Moreover, fumonisin B1 treatment decreased the levels of all measured ceramide species in wild-type LASS5, as well as in the Lag1p motif mutant overexpressing cells (Fig. 6). These data indicate that, in HeLa cells, de novo synthesis of C16 ceramide is likely to be tightly regulated.

**DISCUSSION**

The current study addresses the functional significance of the Lag1p motif of LASS proteins. The Lag1p motif has been recognized as a stretch of amino acids that is characteristically conserved among LASS/Lag homologues (23), but
until now there were no reports for its functional significance. We have shown that six of eight mutated conserved amino acids in the Lag1p motif appeared to be essential to the function of LASS1, because their substitution led to complete loss of enzyme activity in cells and in vitro. The two remaining mutants S193A and K220L did not significantly affect the activity. Moreover, three amino acid point mutations in the Lag1p motif of the human LASS5, introduced at the same positions as the mutations in LASS1, had the same effect on the activity of LASS5 as they had on LASS1. In addition, our results show that LASS1 cannot use a cis-C18:1 fatty acid CoA as a substrate and that LASS1, LASS5, as well as the active mutants are sensitive to fumonisin B1 in HeLa cells. These results show for the first time that the Lag1p motif is required for (dihydro)ceramide synthase activity of LASS proteins.

The hypothesis that the Lag1p motif is functionally important is probably also true for all LASS/Lag proteins, because they share the same conserved amino acids in the motif. The loss of function of the Lag1p motif mutants in LASS/Lag homologues from different species supports this hypothesis. In our study, mutations of histidines or aspartates in the Lag1p motif of mouse LASS1 or human LASS5 had a negative effect on the enzyme activity of these proteins. In support of our observations, a recent study by Kageyama-Yahara and Riezman (27) reports that yeast Lag1 with mutated histidines or aspartates in its Lag1p motif loses its ability to complement the lethality of a Lag1⁰ deletion mutant. In the same yeast study, the Lag1p motif mutations did not affect the membrane topology of Lag1. Interestingly, LASS/Lag proteins can tolerate only conservative substitution at leucines in their Lag1p motifs. We showed that mouse LASS1 lost its enzyme activity when Leu-183 was changed to glutamate (Figs. 2 and 3), whereas Kageyama-Yahara and Riezman (27) report that yeast Lag1 with mutated histidines or aspartates in its Lag1p motif loses its ability to complement the lethality of a Lag1⁰ deletion mutant when the leucine at the same position is altered to alanine. Moreover, only conserved substitution at Leu-254 in the Lag1p motif of the human LASS5 was tolerated. When this leucine was mutated to glutamate, LASS5 lost activity, whereas the bulk of C16 ceramide may not be contributed by the other enzymes of the sphingolipid pathway. Of note is that overexpression of LASS1 and LASS5 in cells had different impacts on C18 and C16 ceramides, respectively (Figs. 2 and 6). Although overexpression of LASS1 resulted in a ~10-fold increase of C18 and C18:1 ceramide compared with the control, the overexpression of LASS5 led only to a modest increase (~25%) of C16 or C14 ceramide species. In HeLa cells, the basal level of C16 ceramide was ~10 times higher than the basal level of C18 ceramide (Figs. 2A and 6A). Therefore, the same amount of increase in C16 or C18 ceramide mass would have a different impact on the fold increase of the respective ceramide species.

It is important to note that, in our experiments, the endogenous (dihydro)ceramide synthase activity with C16 fatty acid CoA as substrate was very low (80 pmol/min/mg protein), and at odds with the fact that C16 ceramide is one of the major ceramide species in HeLa cells. These observations suggest that, in HeLa cells, the C16 ceramide level is tightly regulated and that the bulk of C16 ceramide may not be contributed by CoA-dependent (dihydro)ceramide synthase but by some of the other enzymes of the sphingolipid pathway.

Another important point relates to the observation that fumonisin B1 inhibited the production of C18 ceramide in cells (Fig. 4A) in contrast to previously published results (17) sug-
gesting that LASS1 is a fumonisin B₁-insensitive (dihydro)ceramide synthase. Moreover, in this study, the analyses of C16 ceramide levels of HeLa cells overexpressing LASS5 and treated with fumonisin B₁ showed decrease compared with the controls (Fig. 6), which is also in contrast to a previous publication (19) where C16 ceramide levels increased as a result of LASS5 overexpression and fumonisin B₁ treatment. The only obvious difference between the two sets of experiments, in the cases of LASS1 and LASS5 overexpression, is the choice of cell type. In our experiments, we chose HeLa cells where fumonisin B₁ behaved as an inhibitor, whereas the other two studies (17, 19) used human embryonic kidney 293T cells where fumonisin B₁ stimulated C18 or C16 ceramide production, respectively. The fumonisin B₁ concentrations (20 μM) and the duration of the treatments (for 24 h) were the same. It is also possible that a different level of overexpression is the reason behind the observed differences. Most likely, LASS1 and LASS5 are fumonisin B₁-sensitive (dihydro)ceramide synthases, as shown in our measurements of cellular ceramides (Figs. 4 and 6) and in the in vitro results of the study by Venkataraman et al. (17) and Riebeling et al. (19), but it is still possible that fumonisin B₁ influences ceramide synthesis in a more complex way under the specific conditions of a particular cell type, such as human embryonic kidney 293T cells.

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