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# High activity of acid sphingomyelinase in major depression

*Rapid Communication*

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**Summary.** Acid sphingomyelinase (A-SMase) and its reaction product ceramide may play a role in the pathophysiology of depressive disorders and in the therapeutic action of antidepressive drugs. In a prospective case-control study, A-SMase activity was measured in peripheral blood mononuclear cells of 17 patients with a major depressive episode who were free of antidepressant drug therapy for at least 10 days and 8 healthy volunteers. In the patient group, A-SMase activity was correlated to the score ($n = 17$, $r = 0.64$, $P = 0.005$). The patient group exhibited higher A-SMase activity compared to healthy volunteers ($T = 2.09$, $df = 21.33$, $P < 0.05$). In addition, we demonstrate that the antidepressants imipramine and amitriptyline induce a long-term reduction of the activity of A-SMase in cultured cells.

**Keywords:** Major depression, ceramide, acid sphingomyelinase, sphingomyelin, receptor signaling.

## Introduction

Acid sphingomyelinase (A-SMase, EC 3.1.4.12) is a glycoprotein that functions as a lysosomal hydrolase, catalysing the degradation of sphingomyelin to phosphorylcholine and ceramide. Its name refers to the fact that its optimum activity is approximately at pH 5. Several lines of evidence suggest a potential role of A-SMase and its reaction product ceramide in the pathophysiology and treatment of depressive disorders:

(1) Antidepressant drugs such as desipramine and imipramine inhibit the activity of A-SMase (Albouz et al., 1986). Antidepressant drugs do not directly inhibit A-SMase, but interact with binding of A-SMase to the lipid vesicles (Kölzer et al., 2004), resulting in a release of the enzyme from the

membrane and a proteolytic cleavage in the lysosome. The decrease in enzyme activity appeared to be related to the clinical effectiveness of the drugs investigated (Albouz et al., 1986).

(2) Ceramide reversibly alters the function of the dopamine transporter, resulting in a decreased transport of dopamine and an increased transport of serotonin (Riddle et al., 2003). By inhibition of A-SMase, the therapeutic action of antidepressant drugs might result in lower levels of ceramide and thus in a reduced serotonin transport via the dopamine transporter. In addition to a direct effect of antidepressant drugs on serotonin transporters, this indirect effect may contribute to a delayed serotonin reuptake.

(3) Many surface receptors aggregate in rafts, i.e. cholesterol- and sphingolipid-rich membrane microdomains (Simons and Ikonen, 1997). Hydrolysis of sphingomyelin to ceramide changes the composition of rafts and mediates the formation of large ceramide-enriched membrane platforms that serve to cluster receptors, thus facilitating receptor signaling (Grassmé et al., 2001). This general mechanism also might be of relevance for the etiopathogenesis of depression.

(4) A-SMase treatment induces energy-independent endocytosis in macrophages, fibroblasts (Zha et al., 1998) and in artificial membranes (Nurmienen et al., 2002). This might also occur in neuronal cells and might be involved in the uptake of neurotransmitters from the synaptic space.

(5) Sphingolipid-derived products have been shown to regulate several intracellular proteins. Activation of certain cell surface receptors, for instance TNF, IL-1 or CD95 results in stimulation of A-SMase and a release of ceramide (Nishizuka, 1992; Hannun, 1994; Gulbins et al., 1995). Further, ceramide has been shown to regulate the activity of protein kinase C (Tanabe et al., 1998; Johns et al., 1999; Müller et al., 1995), phospholipase $A_2$ (Huwiler et al., 2001; Koumanov et al., 2002), transacylase (Latorre et al., 2003), stress-activated protein kinases (Westwick et al., 1995), kinase substrate of Ras (Zhang et al., 1997), ceramide-activated protein phosphatase (Dobrowsky and Hannun, 1992) and cathepsin D (Heinrich et al., 2000).

(6) Ceramide has been shown to be involved in the regulation of several ion channels, i.e. the potassium channel Kv1.3 (Gulbins et al., 1997) and the Calcium-Release activated Calcium Channel, CRAC (Lepple-Wienhues et al., 1999). It is quite possible that ceramide alters the activity of ion channels in neurons and thereby contributes to the induction of major depression.

Therefore, we tested the hypothesis of an altered activity of A-SMase in patients with major depressive disorder.

## Materials and methods

Patients and healthy volunteers were investigated in a prospective case-control study. The volunteers were free of neurologic and psychiatric disease and did not take any medication (4 males, 4 females, age range 28–52 years, mean $36.6 \pm 8.0$ years). The patient group consisted of 17 patients (5 males, 12 females, age range 33–72 years, mean $48.0 \pm 12.6$ years) with a current major depressive episode according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4$^{th}$ edition) criteria. Comorbidities were allowed. The score of the 17-item Hamilton

Depression Rating Scale (17-HDRS) was between 16 and 28. All patients were free of antidepressant medication for at least 10 days. Both patients and controls were biologically unrelated. The study was approved by the local ethics committee. Written informed consent was obtained from all participants.

20 ml blood was drawn from patients and volunteers. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-density gradient centrifugation. The activity of A-SMase was determined by immunoprecipitation of the enzyme from cell lysates as well as in whole cell lysates as previously described (Grassmé et al., 1997).

Statistical analyses were computed using SPSS for Windows 12.0.1. Data were analyzed in an exploratory manner using two-tailed parametric statistics. Deviations from a normal distribution were determined by the Kolmogorov-Smirnov-test. A $p < 0.05$ was regarded as significant. Mean values are given $\pm$ SD.

Results are means from duplicate determinations. All assays were performed by a scientist who was unaware of the diagnostic assignments of the samples.

## Results

Our data show a high correlation between the activity of A-SMase measured in whole cells and in cell lysates ($n = 25$, $r = 0.99$, $P < 0.001$). Therefore, the further results are reported for A-SMase activity in lysates only. The variance of

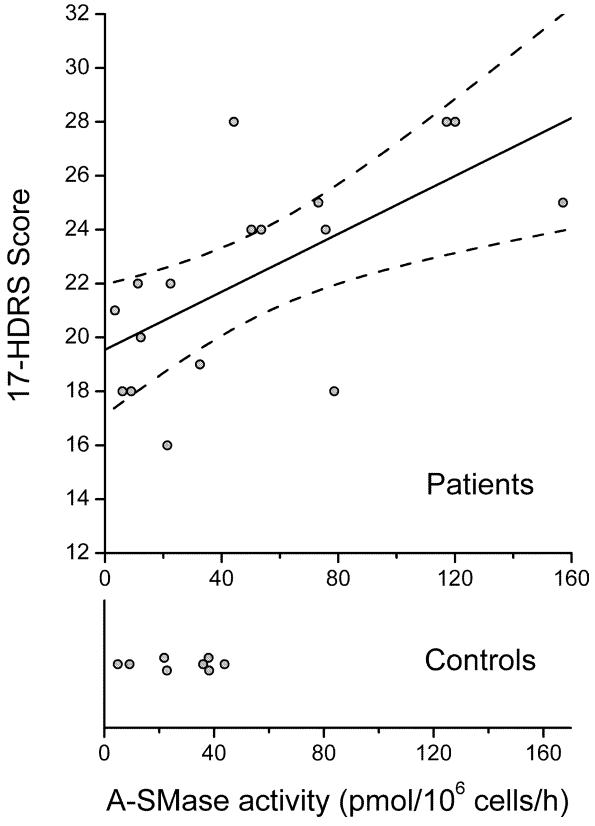


**Fig. 1.** Relationship between A-SMase activity in cell lysates in peripheral blood mononuclear cells and 17-HDRS score. Higher activity of A-SMase was associated with more severe depression ($n = 17$, $r = 0.64$, $P = 0.005$; figure with linear regression line and 95% confidence intervals)

A-SMase activity in patients was higher than in healthy volunteers (Levene test, $F = 6.45$, $P = 0.018$, see also Fig. 1). The results showed a significant increase of the constitute activity of the A-SMase in peripheral blood mononuclear cells of patients with severe major depression, while the A-SMase activity was only weakly increased in patients with a mild form of depression. Overall, the A-SMase activity was higher in patients ($T = 2.09$, $df = 21.33$, $P < 0.05$) and significantly correlated to 17-HDRS scores ($n = 17$, $r = 0.64$, $P = 0.005$). There was no significant relationship between age or sex and A-SMase activity.

Patients age, A-SMase activity and 17-HDRS score were not different from a normal distribution. While sex distribution was matched between healthy volunteers and patients (Fisher's exact Test, $P = 0.19$), the control patients were younger (patients $48.0 \pm 12.6$ years vs controls $36.6 \pm 8.0$ years, $T = 2.32$, df 23, $P = 0.03$).

Treatment of peripheral blood mononuclear cells from healthy control persons with imipramine (10 μM) or amitriptyline (10 μM) resulted in a rapid reduction of the A-SMase activity (Fig. 2). Daily addition of imipramine or amitriptyline was sufficient to keep A-SMase activity reduced over a period of three weeks (Fig. 2). Removal of the drugs resulted in normalization of the A-SMase activity within 5 days. Daily measurements of the A-SMase activity during this time did not show either an overshooting increase or a rebound of A-SMase activity in the reconstitution phase (Fig. 2). Finally, control studies indicate that the activity of the A-SMase did not significantly differ between peripheral blood mononuclear cells freshly isolated or cultured for 30 days (Fig. 2).

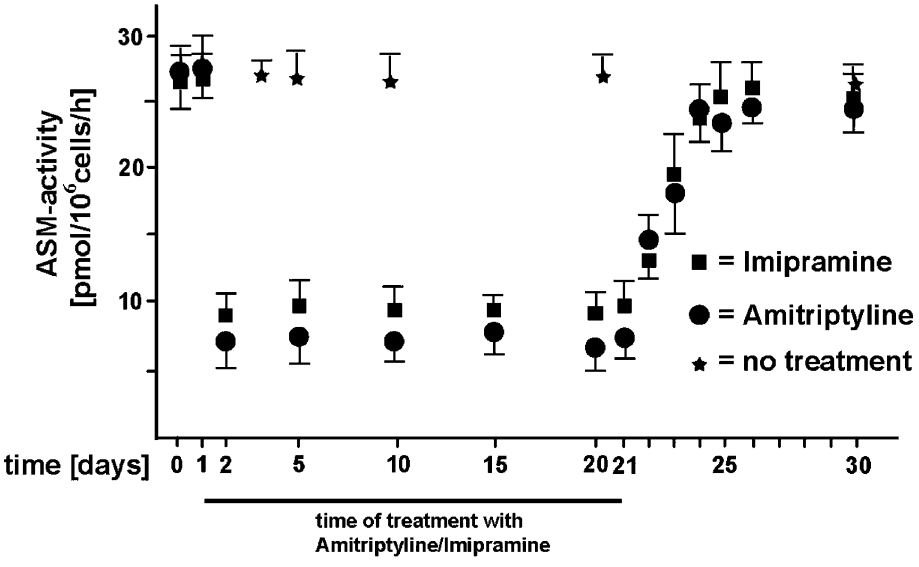


**Fig. 2.** Kinetics of imipramine- and amitriptyline-induced inhibition of A-SMase in peripheral blood mononuclear cells of healthy volunteers. Activity of the A-SMase was determined for 2 days prior and over 20 days after addition of each 10 μM imipramine or amitriptyline. The inhibitors were added daily. In addition, A-SMase activity was determined over time in untreated cells. Shown are the mean ± SD of two independent experiments

## Discussion

Circumstantial evidence links A-SMase activity to major depression, leading us to investigate the activity of this enzyme directly in patients with major depressive disorder. We detected enhanced activity of A-SMase in depressed patients compared to controls and a correlation between the degree of depression as measured with 17-HDRS score and A-SMase activity. These results were not explained by antidepressant medication or by differences in age or sex distribution. Only patients who were free of antidepressant drug therapy for at least 10 days were included in this study. Control experiments confirm that this time frame is sufficient to restore A-SMase activity, even after a prolonged treatment. In cell culture experiments, administration of antidepressant drugs led to reduced A-SMase activity (Albouz et al., 1986; Kölzer et al., 2004), which is confirmed by the present study. The higher A-SMase activity in the patient group indirectly confirms that the patients were free of antidepressant drugs. It is unlikely that previous antidepressant drug therapy had upregulated A-SMase in depressed patients, since we were not able to show an overshooting A-SMase activity after removal of the drugs *in vitro*. Control subjects were younger compared to the patient group. However, there was no correlation between age and A-SMase activity. Furthermore, while the A-SMase activity was higher in patients with a more severe depression, mean age was not different in these patients.

There is probably a close correlation between the activity of A-SMase in peripheral blood mononuclear cells and brain tissue: In Niemann-Pick disease low A-SMase activity has been found both in the brain (Besley and Elleder, 1986) as well as in peripheral blood (Grassmé et al., 2001). Furthermore, drugs like desipramine reduce A-SMase activity in brain tissue (Sakuragawa et al., 1977) as well as in peripheral blood mononuclear cells as described in the present article. Though not proven, it is therefore quite possible that A-SMase activity in major depression is not only enhanced in peripheral blood mononuclear cells but also in brain tissue.

The pathophysiological significance of enhanced activity of A-SMase in major depression remains to be elucidated. However, even today there is evidence that A-SMase is a missing link explaining previously published molecular phenomena in depression und may thus play a central role in the pathophysiology of major depression.

Ceramide activates phospholipase $A_2$ (Huwiler et al., 2001; Koumanov et al., 2002) and stimulates a phospholipase $A_2$ with transacylase activity (Latorre et al., 2003; Abe and Shayman, 1998). Furthermore, ceramide 1-phosphate is a direct activator of cytosolic phospholipase $A_2$ (Pettus et al., 2003). Ceramide alters the fatty acid specificity of phospholipase $A_2$ towards arachidonic acid (Koumanov et al., 2002). Given the enhanced activity of A-SMase, these effects of ceramide and ceramide 1-phosphate should result in an increased activity of phospholipase $A_2$ in major depression and a relative or absolute increase in the ratio of omega 6 to omega 3 fatty acids. Indeed, increased levels of serum phospholipase $A_2$ have been found in mood disorders (Noponen et al., 1993). Lipid-dependent signalling mechanisms involving phospholipase $A_2$ are altered

in depressed patients (Ross et al., 2004). A number of studies have investigated the metabolism and therapeutic effects of polyunsaturated fatty acids in major depression showing changes in brain phospholipid metabolism and increased ratio of omega 6 to omega-3 fatty acids (Adams et al., 1996) in mood disorders. Together, there is evidence of increased activity of phospholipase $A_2$ in mood disorders, which might be caused and/or enhanced by higher activity of A-SMase.

Ceramide differentially regulates protein kinase C isoforms (Tanabe et al., 1998; Johns et al., 1999) with an inhibition of PKC-alpha and an activation of PKC-zeta (Müller et al., 1995). Enhanced activity of A-SMase should therefore result in reduced activity of at least some protein kinase C isoforms in major depression. Indeed, decreased as well as increased activities of protein kinase C have been reported in depression (Akin et al., 2005; Pandey et al., 1997, 2004; Coull et al., 2000). These abnormalities belong to the key disturbances in major depression, which are thought to be corrected by antidepressant drugs. Although speculative, it is possible that the normalization of ceramide upon correction of an increased A-SMase activity also results in a normalization of PKC-activities.

Major depression constitutes a major risk factor for both the development of cardiovascular disease and death after an index myocardial infarction (Musselman et al., 1998). Increased levels of sphingomyelinase might be involved in the pathogenesis of atherosclerosis (Marathe et al., 1999; Augé et al., 2000). Further, a deficiency in omega-3 fatty acids might explain cardiovascular abnormalities in depression (Severus et al., 1999) and an increased activity of sphingomyelinase might thus indirectly contribute to the association between depression and cardiovascular disease via ceramide-induced activation of phospholipase $A_2$ and transacylase.

Taken together, these preliminary data might enhance our understanding of the pathophysiology and pathobiochemistry of major depressive disorder. Compared to control subjects, A-SMase activity is higher in patients with severe major depression and A-SMase activity correlates with the severity of depression. Via ceramide production, enhanced activity of A-SMase may have broad consequences for synaptic transmission and more specifically might increase serotonin uptake via the dopamine transporter. Thus, an inhibition of A-SMase and ceramide-release may finally result in an increase of the serotonin-concentration in the synaptic space. Enhanced activity of A-SMase might be the missing link explaining previously described abnormalities in depression such as altered activity of protein kinase C, increased activity of phospholipase $A_2$ and changes in omega 6 to omega 3 fatty acids. Furthermore, our results might explain the link between depression and cardiovascular disease. Tricyclic antidepressant drugs might normalize the enhanced activity of A-SMase in depressed patients via proteolytic degradation of A-SMase. Finally, the results of our study suggest a novel molecular target for antidepressant drug therapy.

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## References


Abe A, Shayman JA (1998) Purification and characterization of 1-*O*-acylceramide synthase, a novel phospholipase $A_2$ with transacylase activity. J Biol Chem 273: 8467–8474

Adams PB, Lawson S, Sanigorski A, Sinclair AJ (1996) Arachidonic acid to eicosapentaenoic acid ratio in blood correlates positively with clinical symptoms of depression. Lipids 31 (Suppl): S157–S161

Akin D, Manier DH, Sanders-Bush E, Shelton RC (2005) Signal transduction abnormalities in melancholic depression. Int J Neuropsychopharmacol 8: 5–16

Albouz S, Le Saux F, Wenger D, Hauw JJ, Baumann N (1986) Modifications of sphingomyelin and phosphatidylcholine metabolism by tricyclic antidepressants and phenothiazines. Life Sci 38: 357–363

Augé N, Nègre-Salvayre A, Salvayre R, Levade T (2000) Sphingomyelin metabolites in vascular cell signaling and atherogenesis. Prog Lipid Res 39: 207–229

Besley GT, Elleder M (1986) Enzyme activities and phospholipid storage patterns in brain and spleen samples from Niemann-Pick disease variants: a comparison of neuropathic and non-neuropathic forms. J Inherit Metab Dis 9: 59–71

Coull MA, Lowther S, Katona CLE, Horton RW (2000) Altered brain protein kinase C in depression: a post-mortem study. Eur Neuropsychopharmacol 10: 283–288

Dobrowsky RT, Hannun YA (1992) Ceramide stimulates a cytosolic protein phosphatase. J Biol Chem 267: 5048–5051

Grassmé H, Gulbins E, Brenner B, Ferlinz K, Sandhoff K, Harzer K, Lang F, Meyer TF (1997) Acidic sphingomyelinase mediates entry of N. gonorrhoeae into nonphagocytic cells. Cell 91: 605–615

Grassmé H, Jekle A, Riehle A, Schwarz H, Berger J, Sandhoff K, Kolesnick R, Gulbins E (2001) CD95 signaling via ceramide-rich membrane rafts. J Biol Chem 276: 20589–20596

Gulbins E, Bissonnette R, Mahboubi A, Martin S, Nishioka W, Brunner T, Baier G, Baier-Bitterlich G, Byrd C, Lang F, Kolesnick R, Altman A, Green D (1995) FAS-induced apoptosis is mediated via a ceramide-initiated RAS signaling pathway. Immunity 2: 341–351

Gulbins E, Szabo I, Baltzer K, Lang F (1997) Ceramide-induced inhibition of T lymphocyte voltage-gated potassium channel is mediated by tyrosine kinases. Proc Natl Acad Sci USA 94: 7661–7666

Hannun YA (1994) The sphingomyelin cycle and the second messenger function of ceramide. J Biol Chem 269: 3125–3128

Heinrich M, Wickel M, Winoto-Morbach S, Schneider-Brachert W, Weber T, Brunner J, Saftig P, Peters C, Krönke M, Schütze S (2000) Ceramide as an activator lipid of cathepsin D. Adv Exp Med Biol 477: 305–315

Huwiler A, Johansen B, Skarstad A, Pfeilschifter J (2001) Ceramide binds to the CaLB domain of cytosolic phospholipase $A_2$ and facilitates its membrane docking and arachidonic acid release. FASEB J 15: 7–9

Johns DG, Jin JS, Wilde DW, Webb RC (1999) Ceramide-induced vasorelaxation: an inhibitory action on protein kinase C. Gen Pharmacol 33: 415–421

Kölzer M, Werth N, Sandhoff K (2004) Interactions of acid sphingomyelinase and lipid bilayers in the presence of the tricyclic antidepressant desipramine. FEBS Lett 559: 96–98

Koumanov KS, Momchilova AB, Quinn PJ, Wolf C (2002) Ceramides increase the activity of the secretory phospholipase $A_2$ and alter its fatty acid specificity. Biochem J 363: 45–51

Latorre E, Collado MP, Fernández I, Aragonés MD, Catalán RE (2003) Signaling events mediating activation of brain ethanolamine plasmalogen hydrolysis by ceramide. Eur J Biochem 270: 36–46

Lepple-Wienhues A, Belka C, Laun T, Jekle A, Walter B, Wieland U, Welz M, Heil L, Kun J, Busch G, Weller M, Bamberg M, Gulbins E, Lang F (1999) Stimulation of CD95 (Fas) blocks T lymphocyte calcium channels through sphingomyelinase and sphingolipids. Proc Natl Acad Sci USA 96: 13795–13800

Marathe S, Kuriakose G, Williams KJ, Tabas I (1999) Sphingomyelinase, an enzyme implicated in atherogenesis, is present in atherosclerotic lesions and binds to specific components of the subendothelial extracellular matrix. Arterioscler Thromb Vasc Biol 19: 2648–2658

Müller G, Ayoub M, Storz P, Rennecke J, Fabbro D, Pfizenmaier K (1995) PKC ζ is a molecular switch in signal transduction of TNF-α, bifunctionally regulated by ceramide and arachidonic acid. EMBO J 14: 1961–1969

Musselman DL, Evans DL, Nemeroff CB (1998) The relationship of depression to cardiovascular disease: epidemiology, biology, and treatment. Arch Gen Psychiatry 55: 580–592

Nishizuka Y (1992) Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 258: 607–614

Noponen M, Sanfilipo M, Samanich K, Ryer H, Ko G, Angrist B, Wolkin A, Duncan E, Rotrosen J (1993) Elevated $PLA_2$ activity in schizophrenics and other psychiatric patients. Biol Psychiatry 34: 641–649

Nurmienen TA, Holopainen JM, Zhao H, Kinnunen PKJ (2002) Observation of topical catalysis by sphingomyelinase coupled to microspheres. J Am Chem Soc 124: 12129–12134

Pandey GN, Dwivedi Y, Pandey SC, Conley RR, Roberts RC, Tamminga CA (1997) Protein kinase C in the postmortem brain of teenage suicide victims. Neurosci Lett 228: 111–114

Pandey GN, Dwivedi Y, Rizavi HS, Ren X, Conley RR (2004) Decreased catalytic activity and expression of protein kinase C isozymes in teenage suicide victims: a postmortem brain study. Arch Gen Psychiatry 61: 685–693

Pettus BJ, Bielawska A, Spiegel S, Roddy P, Hannun YA, Chalfant CE (2003) Ceramide kinase mediates cytokine- and calcium ionophore-induced arachidonic acid release. J Biol Chem 278: 38206–38213

Riddle EL, Rau KS, Topham MK, Hanson GR, Fleckenstein AE (2003) Ceramide-induced alterations in dopamine transporter function. Eur J Pharmacol 458: 31–36

Ross BM, Ward P, Glen I (2004) Delayed vasodilatory response to methylnicotinate in patients with unipolar depressive disorder. J Affect Disord 82: 285–290

Sakuragawa N, Sakuragaw M, Kuwabara T, Pentchev PG, Barranger JA, Brady RO (1977) Niemann-Pick disease experimental model: sphingomyelinase reduction induced by AY-9944. Science 196: 317–319

Severus WE, Ahrens B, Stoll AL (1999) Omega-3 fatty acids – the missing link? Arch Gen Psychiatry 56: 380–381

Simons K, Ikonen E (1997) Functional rafts in cell membranes. Nature 387: 569–572

Tanabe F, Cui SH, Ito M (1998) Ceramide promotes calpain-mediated proteolysis of protein kinase C β in murine polymorphonuclear leukocytes. Biochem Biophys Res Commun 242: 129–133

Westwick JK, Bielawska AE, Dbaibo G, Hannun YA, Brenner DA (1995) Ceramide activates the stress-activated protein kinases. J Biol Chem 270: 22689–22692

Zha X, Pierini LM, Leopold PL, Skiba PJ, Tabas I, Maxfield FR (1998) Sphingomyelinase treatment induces ATP-independent endocytosis. J Cell Biol 140: 39–47

Zhang Y, Yao B, Delikat S, Bayoumy S, Lin XH, Basu S, McGinley M, Chan-Hui PY, Lichenstein H, Kolesnick R (1997) Kinase suppressor of Ras is ceramide-activated protein kinase. Cell 89: 63–72

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