Original Paper

Role of Janus-Kinases in Major Depressive Disorder

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Key Words

Janus kinases (Jaks) • Neuronal stem cells • Major depression • Acid sphingomyelinase

Abstract

Background/Aims: Major depressive disorder is a severe, common and often chronic disease with a significant mortality due to suicide. The pathogenesis of major depression is still unknown. It is assumed that a reduction of neurogenesis in the hippocampus plays an important role in the development of major depressive disorder. However, the mechanisms that control proliferation of neuronal stem cells in the hippocampus require definition. Here, we investigated the role of Janus-Kinase 3 (Jak-3) for stress-induced inhibition of neurogenesis and the induction of major depression symptoms in mice. **Methods:** Stress was induced by the application of glucocorticosterone. Brain sections were stained with phospho-specific antibodies and analysed by confocal microscopy to measure phosphorylation of Jak-3 specifically in the hippocampus. Jak-3 inhibitors and the antidepressant amitriptyline were applied to counteract stress. The effects of the inhibitors were determined by a set of behavioural tests and analysis of Jak-3 phosphorylation in brain sections. Acid sphingomyelinase-deficient mice were employed to test whether Jak3 is downstream of ceramide. **Results:** The data show that stress reduces neurogenesis, which is restored by simultaneous application of Jak-3 inhibitors. Inhibition of neurogenesis correlated with an anxious-depressive behaviour that was also normalized upon application of a Jak-3-inhibitor. Confocal microscopy data revealed that stress triggers a phosphorylation and thereby activation of Jak-3 in the hippocampus. Amitriptyline, a commonly used antidepressant that blocks the acid sphingomyelinase, or acid sphingomyelinase-deficiency reduced stress-induced phosphorylation of Jak-3. **Conclusion:** Our data show that Jak-3 is activated by stress at least partially via the acid sphingomyelinase and is involved in the mediation of stress-induced major depression.

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Introduction

Major depressive disorder is a severe and chronic disease, with up to 10% of the population being affected at least once in their lifetime [1, 2]. Patients with major depressive disorder suffer from a variety of symptoms including depressed mood, melancholia, inability to feel, loss of interest, anhedonia, fear, insomnia, concentration deficits, but also several somatic symptoms [1-3].

The pathogenesis of major depression is still unknown and requires definition. Unpredictable and chronic stress can increase the risk of developing major depression. Further an increase of glucocorticoids seems to be important in the pathogenesis of neurogenesis in some, but not all patients with major depressive disorder. It has been suggested that a decreased concentration of monoaminergic neurotransmitters in the synaptic space causes major depression [3]. However, this fails to explain why antidepressants that rapidly prevent uptake, and therefore immediately increase the concentration of monoaminergic neurotransmitters in the synaptic space, particularly serotonin and norepinephrine, only act after 2-4 weeks of treatment. Further, some antidepressants such as Tianeptine even induce an uptake of neurotransmitters [4]. Thus, newer concepts have suggested that major depression is caused by a decrease of neurogenesis in the hippocampus and possibly also the frontal brain [5-10]. These regions contain neuronal stem cells and approximately 700 new neurons are born in the hippocampus daily [5-8]. Antidepressants increase the proliferation of neurons and reverse the decrease of size in the hippocampus observed in many patients with major depression [8, 9]. Reduced neurogenesis might result in a rarefaction and finally failure of neuronal networks in the hippocampus and thereby the symptoms of major depressive disorder. However, the reason for reduced neurogenesis in patients with major depressive disorder still requires definition.

We have recently shown that the acid sphingomyelinase/ceramide system is targeted by antidepressants [10]. The acid sphingomyelinase is an ubiquitously-expressed enzyme that converts sphingomyelin to ceramide [11]. Ceramide regulates cell signaling by re-organisation of proteins in the cell membrane and has been shown to be involved in many forms of stress and programmed cell death [11-17]. However, stress induces symptoms of major depressive disorder even in mice lacking the acid sphingomyelinase suggesting that ceramide does not directly mediate stress. In contrast to wildtype mice, acid sphingomyelinase-deficient mice fail to respond to antidepressants. This suggests that ceramide functions as a negative regulator of neurogenesis and that a reduction of ceramide by antidepressants reduces the sum of negative stimuli on neurogenesis in stressed animals allowing the recovery of a normal neurogenesis. Downstream targets of the acid sphingomyelinase/ceramide system in neurogenesis are presently poorly characterized.

Here, we investigated the role of Janus Kinases (Jaks) in major depressive disorder and whether Jaks are downstream of the acid sphingomyelinase/ceramide system in the hippocampus. It is presently unknown whether Jaks are involved in neurogenesis *in vivo* and in major depressive disorder. Jaks respond to several cytokine receptors, for instance the interleukin (IL)-6 and -12-receptors [18]. Binding of the respective ligands/cytokines alters the conformation of these cytokine receptors, which results in transphosphorylation and activation of Jaks that are bound to the receptor [18]. Active Jaks phosphorylate their receptors, which then bind Stat-proteins (signal transducer and activators of transcription). Their phosphorylation leads to dissociation from the receptor and migration into the nucleus to regulate protein biosynthesis [18, 19]. Studies in cultured neurons revealed that pharmacological inhibition of Jak-3 inhibitor promotes neurogenesis [20]. However, whether these *in vitro* data also apply to the *in vivo* situation is presently unknown.

In the present study we investigated the role of Jak-3 in glucocorticoid-induced major depression. We analysed whether stress induces an alteration of Jak-3 activity, whether Jak-3 is involved in neurogenesis *in vivo* in the hippocampus and whether Jak-3 functions downstream of the acid sphingomyelinase/ceramide system.

Materials and Methods

Chemicals and antibodies

All reagents were from Sigma-Aldrich, Deisenhofen, if not otherwise noted. The Jak-3 inhibitor IV (sc 295217) was from Santa Cruz Inc., anti-bromodeoxyuridine (BrdU) antibodies were from Millipore, BrdU from Roche. Anti-phospho-Jak-3 and anti-phospho-Stat-3 antibodies were from Cell Signalling. Fluorescence-labeled antibodies were from ImmunoResearch. Phosphate buffered salt solution consisted of 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄ and 1.8 mM KH₂PO₄. HEPES-buffered saline solution (H/S) consisted of 20 mM HEPES, 132 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 0.7 mM MgCl₂, 0.8 mM MgSO₄, pH 7.4.

Mice

C57BL/6 wild type or acid sphingomyelinase-deficient mice [21] were treated for 14 days with 250 mg/L glucocorticosterone via the drinking water [10]. To this end, glucocorticosterone was dissolved in dimethylsulfoxide (DMSO) at 125 mg/mL and then further diluted in 0.9% NaCl, which served as drinking water. Jak-3 was inhibited *in vivo* by i.p. injection of the Jak-3 inhibitor IV at 40 mg/kg twice daily for up to 14 days. The inhibitor was dissolved in DMSO and 1:10 diluted in 0.9% NaCl solution. Amitriptyline was applied in the drinking water at 120 mg/L. All animal experiments were performed with permission of the Regierungspraesidium Düsseldorf and the Institutional Animal Care and Use Committee, Cincinnati.

Behavioural tests

Dark-Light Box. Mice were individually placed in a dark and safe compartment, which is connected via 5×5 cm aperture with rounded corners with a brightly illuminated, open, and thus aversive area of the same size. Each mouse was released in the dark compartment, observed for 5 min and the time the mouse was outside in the open area was recorded.

Novelty-suppressed feeding test or latency to feed test. Mice were fasted for 24 hrs. The test then measured the time during which the mice explore a new environment before they began eating.

Bromodeoxyuridin (BrdU)-staining. Mice were injected 4 times (every 2 hrs) with BrdU, sacrificed 24 hrs after the 1st injection, brains were placed in Tissue-Tek and shock-frozen in liquid nitrogen. Sections were performed on a Leica cryotome, dried on air for 5 min and fixed for 10 min in ice-cold acetone. Samples were washed twice in PBS, incubated for 2 h with 50% formamide in 300 mM NaCl and 30 mM saline-sodium citrate (SSC; pH 7.0) at 65°C, washed twice in saline sodium citrate (SSC) buffer and then incubated for 30 min at 37°C with 2 M HCl. These steps serve to denature the DNA. The samples were then washed in PBS, neutralized for 10 min with 0.1 M borate buffer (pH 8.5), washed, and incubated with 0.05% Tween 20 and 5% FCS in PBS (pH 7.4) to block unspecific binding sites. The samples were incubated with 5 µg/mL anti-BrdU antibodies (Roche) for 45 min at 22°C, washed, labeled with Cy3-coupled F(ab)₂ anti-mouse IgG (Jackson ImmunoResearch), washed and embedded in Mowiol. Serial sections were counted for BrdU-positive cells.

Phospho-Jak-3- and phospho-Stat-3-stainings. Frozen sections of the brains were dried and fixed as above. Sections were washed 3-times in PBS, blocked in H/S + 5% FCS, washed once in PBS and incubated with anti-phospho-Jak-3 or anti-phospho-Stat-3 antibodies (each diluted 1:100 in H/S + 1% FCS) for 45 min at 22°C. Samples were washed 3 times, each 5 min in PBS + 0.05% Tween 20, and once in PBS. They were then stained with secondary Cy3-labeled donkey F(ab)₂- anti-rabbit fragments for 45 min at 22°C. The samples were washed again 3-times each 5 min PBS + 0.05% Tween 20 and once in PBS, embedded in Mowiol and analyzed by confocal microscopy using a Leica TCS DMIRE.

Statistics

Data were examined with analysis of variance (ANOVA) and post hoc tests. A *p* value of 0.05 or less (two-tailed) was considered to represent statistical significant differences.

Results

Jak-3 inhibition improves behaviour of stressed mice

In order to test whether Janus-kinases (Jaks) play a role in stress-induced major depressive disorder, we treated mice with glucocorticosterone (250 mg/L) via the drinking

water for 14 days. High doses of glucocorticosterone have been previously shown to induce depressive behaviour (10). The following groups were investigated:

1. Mice that only received glucocorticosterone,
2. mice that were i.p. injected with the Jak-3 inhibitor IV twice daily starting 3 days after initiation of the glucocorticosterone treatment,
3. mice that only received Jak-3 inhibitor IV,
4. mice that were treated with glucocorticosterone + i.p. injection of 10% DMSO in 0.9% NaCl, the solvent of the JAK-3 IV inhibitor (these mice were more stressed than mice that only received glucocorticosterone, since the injection also constitutes a short, but severe stress),
5. mice that received amitriptyline in the drinking water,
6. mice that were treated simultaneously with amitriptyline and glucocorticosterone in the drinking water (the amitriptyline was started as above 3 days after the glucocorticosterone treatment was initiated),
7. untreated mice.

Mice were randomly distributed to the groups. There were no differences in the age and sex distribution between the groups. The investigator performing the behavioural test was blinded to the treatment of the mice. Behaviour was measured after 9-10 days of treatment with the Jak-3 inhibitor IV or the corresponding time point in the other groups.

The Dark-Light-Box tests revealed that glucocorticosterone-treated or glucocorticosterone + solvent-treated mice remained outside the dark box for a much shorter time than untreated mice (Fig. 1A). Injection of the Jak-3 inhibitor IV markedly improved the behaviour and mice treated with this inhibitor were in the light box almost as long as untreated control mice (Fig. 1A). A similar result was obtained in the Latency to Feed test: Stress, either only glucocorticosterone or the combination of glucocorticosterone + solvent injection, induced a severe anxious and depressive behaviour and these mice required much more time before they started to eat in a new environment after a 24 hr fasting period (Fig. 1B). Treatment of the mice with the Jak-3 inhibitor IV normalized behaviour of stressed mice (Fig. 1B); the Jak-3 inhibitor did not alter the behaviour of not-stressed mice.

Fig. 1. Inhibition of Jak-3 improves depressive-anxious behaviour of stressed mice. Wild type mice were stressed or left unstressed and treated as indicated. The behaviour of the mice was analyzed in the "Dark-Light-Box-Test" (A) or the "Latency to Feed-Test" (B). Both tests reveal an anxious-depressive behaviour in stressed mice that is improved or normalized upon treatment with the Jak-3 inhibitor IV. Displayed are the mean $\pm$ SD of 5 mice per group, * p <0.05, ANOVA.

Fig. 2. Jak-3 inhibition prevents stress-reduced hippocampal neurogenesis. Wild type mice were treated as indicated, intraperitoneally injected 4-times with bromodeoxyuridine (BrdU, 2 mg) and 16 hrs later sacrificed. Brains were removed, shock-frozen, serial sections were performed and stained with Cy3-labelled anti-BrdU-antibodies. The number of BrdU-positive neurons was counted in serial sections and extrapolated to the number in the whole hippocampus (A). The studies reveal a marked inhibition of neurogenesis upon stress, which is prevented by application of the Jak-3 inhibitor IV. Displayed are the mean $\pm$ SD of 5 mice per group, * $p < 0.05$, ANOVA, in panel A or representative confocal microscopy studies of the hippocampus sections in panel B.

These data show that inhibition of Jak-3 normalizes depressive and anxious behaviour of stressed mice.

Jak-3 inhibitors increase hippocampal neurogenesis

In the next experimental series we investigated whether Jak-3-inhibitor IV also influences hippocampal neurogenesis. Reduced neurogenesis is a hallmark of major depression induced by stress [7, 9, 10]. To this end, we injected mice 4 times daily with BrdU, sacrificed the mice 24 hrs after the 1st injection and immuno-stained the sections for BrdU in the hippocampus. This method allows counting the number of proliferating cells. The number of BrdU-positive nuclei in the hippocampus was counted in serial sections and reflects the proliferation in the hippocampus. These investigations revealed a marked reduction of neurogenesis in the stressed mice, which was corrected by treatment with the Jak-3 inhibitor IV (Fig. 2A and 2B).

Stress induces phosphorylation of Jak-3

In the next series, we determined whether stress has a direct impact on the phosphorylation status of Jak-3 (Fig. 3). Phosphorylation of Jaks is a measurement of the activity of these kinases [19]. Further, we analysed whether Stat-3, a target of Jaks [19], is phosphorylated and thereby activated after stress and whether the Jak-3 inhibitor prevents this phosphorylation (Fig. 3). These data demonstrate that stress induces phosphorylation of Jak-3 in the hippocampus, which is prevented by treatment with the Jak-3 inhibitor IV (Fig. 3A). Phosphorylation of Stat-3 was not altered after stress, nor after treatment with the Jak-3 inhibitor, indicating that Stat-3 does not serve as target of Jak-3 after stress in hippocampal neurons (Fig. 3B)

Regulation of Jak-3 activity by amitriptyline

In the last series of experiments we investigated whether application of amitriptyline, a commonly used antidepressant drug, regulates the phosphorylation of Jak-3. To this end, we determined the phosphorylation of Jak-3 in hippocampus-sections of mice that were

Fig. 3. Stress induces phosphorylation of Jak-3, but not of Stat-3. Stress induces phosphorylation of Jak-3 (A), while the phosphorylation of Stat-3 is not altered upon glucocorticoid-stress (B). Treatment of the mice with the Jak-3 inhibitor IV prevents stress-induced phosphorylation of Jak-3. Shown are representative confocal microscopy studies of the hippocampus sections stained with Cy3-labelled anti-phospho-Jak-3 or anti-phospho-Stat-3 antibodies, respectively. 5 mice per group were analyzed. Phosphorylated neurons in the neurogenic zone are indicated by arrows.

stressed with glucocorticosterone or treated with glucocorticosterone + amitriptyline (Fig. 4A). Control mice received only amitriptyline or were left untreated. Again, stress induced a phosphorylation of Jak-3 (Fig. 4A). Application of amitriptyline markedly reduced the effect of stress on Jak-3-phosphorylation and thereby activity of Jak-3 (Fig. 4A). Amitriptyline was without effect on the low basal phosphorylation of Jak-3 in the hippocampus (Fig. 4A). We have previously shown that the antidepressant effect of amitriptyline is mediated by an inhibition of the acid sphingomyelinase and a reduction of ceramide [10]. To test whether amitriptyline reduces the phosphorylation and activity of Jak-3 via inhibition of the acid sphingomyelinase, we stressed acid sphingomyelinase-deficient mice with glucocorticosterone and tested whether amitriptyline still reduces phosphorylation of Jak-3 or not. Surprisingly, the data demonstrate that acid sphingomyelinase-deficiency itself reduced phosphorylation of Jak-3 upon stress. Amitriptyline, however, had no additional effect on the phosphorylation of Jak-3 (Fig. 4B), indicating that amitriptyline regulates Jak-3 via the acid sphingomyelinase.

Discussion

The data of the present study demonstrate that (i) Jak-3 is a stress-activated molecule, (ii) Jak-3 is critically involved in the regulation of hippocampal neurogenesis under stress,

Fig. 4. Pharmacological or genetic blockade of the acid sphingomyelinase prevents Jak-3 phosphorylation after stress. Stress induces phosphorylation of Jak-3 (A), an effect that is reduced, but not absent, in acid sphingomyelinase-deficient mice exposed to stress (B). Likewise, treatment of wild type mice with the antidepressive drug amitriptyline reduces stress-induced Jak-3 phosphorylation (A), while amitriptyline was without effect in acid sphingomyelinase-deficient mice (B). Jak-3-phosphorylation is mainly detected in the neurogenic zone of the hippocampus. Displayed are representative confocal microscopy studies from brain sections that were stained with Cy3-labelled anti-phospho-JAK-3 antibodies. We analysed serial sections from 5 mice per group. Phosphorylated neurons in the neurogenetic zone are indicated by arrows.

(iii) direct inhibition of Jak-3 by a Jak-3 inhibitor improves stress-induced depressive behaviour and normalizes neurogenesis, (iv) the acid sphingomyelinase mediates (indirectly) phosphorylation of Jak-3 upon stress and (v) amitriptyline regulates these effects by inhibition of the acid sphingomyelinase in the hippocampus of stressed mice.

At present, the role of Jaks in major depression is unknown. A previous study demonstrated a role of Jak-2 in drug-induced damage of the striatum [22]. This study revealed that inhibition of Jak-2 reduced neuronal damage and the formation of scars after quinolinic acid treatment [22]. This model is completely unrelated to major depression, but the role of Jak-2 is analogous to that of Jak-3 in the present study.

A further study on the role of Jaks in neuronal proliferation used cultured neurons *in vitro* [20]. This study demonstrated that inhibition of Jak-3 induces proliferation of neurons *in vitro* [20], consistent with the present *in vivo* data.

The role of acid sphingomyelinase in the regulation of Jaks in neuronal stem cells has not been investigated to date. Our studies on wild type and acid sphingomyelinase-deficient

mice demonstrate that activation of Jak-3 by stress is at least partly mediated by the acid sphingomyelinase and its product ceramide. Since phosphorylation of Jak-3 is only reduced, but not abrogated by genetic deficiency of the acid sphingomyelinase, it must be assumed that Jak-3 is also targeted by stress via pathways independent of the acid sphingomyelinase.

The effect of the acid sphingomyelinase on Jak-3 is consistent with the observation that treatment with amitriptyline reduced the phosphorylation of Jak-3 in stressed mice. Amitriptyline is a functional inhibitor of the acid sphingomyelinase, which competes with the enzyme for binding to the inner lysosomal membrane [23]. The release of the acid sphingomyelinase from the membrane results in proteolytic cleavage of the enzyme and thereby in a reduction of its cellular activity [23, 24]. Amitriptyline and many other tricyclic or structurally similar antidepressant drugs are therefore functional inhibitors of the acid sphingomyelinase [23]. We have previously shown that amitriptyline targets the acid sphingomyelinase, reduces ceramide and thereby increases neurogenesis and improves behaviour of stressed mice [10]. Amitriptyline was without effect on Jak-3 phosphorylation in acid sphingomyelinase-deficient mice consistent with the upstream regulation of Jak-3 by the acid sphingomyelinase. At present it is unclear how the acid sphingomyelinase/ceramide system regulates Jak-3 phosphorylation.

The acid sphingomyelinase/ceramide system seems to be a central checkpoint regulating neurogenesis and stress responses in the hippocampus. Thus, it has been previously shown that inhibition of the acid sphingomyelinase/ceramide systems prevents the effects of stress in the hippocampus [10, 25-27] and functions upstream of stress-activated kinases such as p38Kinase [25].

Our experiments on the role of Stat-3 as a target of the increased Jak-3 activity after stress revealed a negative result indicating that Stat-3 does not play a role in stress-induced effects in the hippocampus. At present it is unknown which one of the six different Stat-proteins responds to Jak-3 activation upon application of stress and which Stat-protein finally regulates neuronal proliferation upon stress. In contrast to present findings on glucocorticoid-induced stress, a recent study demonstrated an activation of Stat-3 in hippocampal neurons after IL-6 stimulation [28]. These data suggest that different forms of stress such as glucocorticoid- or inflammation-triggered stress induce the Jak-Stat system, but different members of this system. However, the final outcome seems to be very similar, i.e. down-regulation of neurogenesis.

The present data indicate a marked effect of the Jak-3 inhibitor IV on stress-induced behaviour and neurogenesis in the hippocampus. At present it is unknown whether Jak-3 inhibitors that were developed and presently investigated to treat rheumatoid arthritis [19] also have an antidepressant effect in humans.

In summary, we have demonstrated that stress induces an activation of Jak-3 resulting in inhibition of neurogenesis and depressive-like behaviour. Direct inhibition of Jak-3 with a Jak-3 inhibitor prevents these effects in stressed mice. Stress activates Jak-3, at least in part, via the acid sphingomyelinase and inhibition of this enzyme using the antidepressant drug amitriptyline reduces Jak-3 phosphorylation and improves behaviour as well as hippocampal neurogenesis. Our data suggest that Jak-3 inhibitors should be investigated for their potential to treat major depressive disorder, although the use of those drugs has to be carefully monitored for potential immunological side effects, since Jak-inhibitors were shown to have severe immunosuppressive side effects [29].

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Disclosure Statement

The authors have no conflict of interests to declare.

References

- 1 Belmaker RH, Agam G: Major depressive disorder. *N Engl J Med* 2008;358:55-68.
- 2 Gulbins E, Walter S, Becker KA, Halmer R, Liu Y, Reichel M, Edwards MJ, Müller CP, Fassbender K, Kornhuber J: A central role for the acid sphingomyelinase/ceramide system in neurogenesis and major depression. *J Neurochem* 2015;134:183-192.
- 3 Hirschfeld RM: History and evolution of the monoamine hypothesis of depression. *J Clin Psychiatry* 2000;61:Suppl 6, 4-6.
- 4 Brink CB, Harvey BH, Brand L: Tianeptine: a novel atypical antidepressant that may provide new insights into the biomolecular basis of depression. *Rec Pat CNS Drug Discov* 2006;1:29-41.
- 5 Amrein I, Isler K, Lipp HP: Comparing adult hippocampal neurogenesis in mammalian species and orders: influence of chronological age and life history stage. *Eur J Neurosci* 2011;34:978-987.
- 6 Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, Tisdale J, Possnert G, Druid H, Frisén J: Neurogenesis in the striatum of the adult human brain. *Cell* 2014;156:1072-1083.
- 7 Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R: Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 2003;301:805-809.
- 8 Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, Boström E, Westerlund I, Vial C, Buchholz BA, Possnert G, Mash DC, Druid H, Frisén J: Dynamics of hippocampal neurogenesis in adult humans. *Cell* 2013;153:1219-1227.
- 9 Sahay A, Hen R: Adult hippocampal neurogenesis in depression. *Nat Neurosci* 2007;10:1110-1115.
- 10 Gulbins E, Palmada M, Reichel M, Lüth A, Böhmer C, Amato D, Müller CP, Tischbirek CH, Groemer TW, Tabatabai G, Becker KA, Tripal P, Staedtler S, Ackermann TF, van Brederode J, Alzheimer C, Weller M, Lang UE, Kleuser B, Grassmé H, Kornhuber J: Acid sphingomyelinase-ceramide system mediates effects of antidepressant drugs. *Nat Med* 2013;19:934-938.
- 11 Gulbins E, Kolesnick R: Raft ceramide in molecular medicine. *Oncogene* 2003;22:7070-7077.
- 12 Grassmé H, Jekle A, Riehle A, Schwarz H, Berger J, Sandhoff K, Kolesnick R, Gulbins E: CD95 signaling via ceramide-rich membrane rafts. *J Biol Chem* 2001;276:20589-20596.
- 13 Grassmé H, Jendrossek V, Riehle A, von Kurthy G, Berger J, Schwarz H, Weller M, Kolesnick R, Gulbins E: Host defense against *Pseudomonas aeruginosa* requires ceramide-rich membrane rafts. *Nat Med* 2003;9:322-330.
- 14 Lang PA, Schenck M, Nicolay JP, Becker JU, Kempe DS, Lupescu A, Koka S, Eisele K, Klarl BA, Rübber H, Schmid KW, Mann K, Hildenbrand S, Heftner H, Huber SM, Wieder T, Erhardt A, Häussinger D, Gulbins E, Lang F: Liver cell death and anemia in Wilson disease involve acid sphingomyelinase and ceramide. *Nat Med* 2007;13:164-70.
- 15 Teichgräber V, Ulrich M, Endlich N, Riethmüller J, Wilker B, De Oliveira-Munding CC, van Heeckeren AM, Barr ML, von Kurthy G, Schmid KW, Weller M, Tümmeler B, Lang F, Grassme H, Döring G, Gulbins E: Ceramide accumulation mediates inflammation, cell death and infection susceptibility in cystic fibrosis. *Nat Med* 2008;14:382-391.
- 16 Tavakoli Tabazavareh S, Seitz A, Jernigan P, Sehl C, Keitsch S, Lang S, Kahl BC, Edwards M, Grassmé H, Gulbins E, Becker KA: Lack of sphingosine causes susceptibility to pulmonary *Staphylococcus aureus* infections in cystic fibrosis. *Cell Physiol Biochem* 2016;38:2094-2102.
- 17 Li X, Gulbins E, Zhang Y: Oxidative stress triggers Ca²⁺-dependent lysosome trafficking and activation of acid sphingomyelinase. *Cell Physiol Biochem* 2012;30:815-826.

- 18 Falus A: Cytokine receptor architecture, structure and genetic assembly. *Immunol Lett* 1995;44:221-223.
- 19 Aittomäki S, Pesu M: Therapeutic targeting of the Jak/STAT pathway. *Basic Clin Pharmacol Toxicol* 2014;114:18-23.
- 20 Kim YH, Chung JI, Woo HG, Jung YS, Lee SH, Moon CH, Suh-Kim H, Baik EJ: Differential regulation of proliferation and differentiation in neural precursor cells by the Jak pathway. *Stem Cells* 2010;28:1816-1828.
- 21 Horinouchi K, Erlich S, Perl DP, Ferlinz K, Bisgaier CL, Sandhoff K, Desnick RJ, Stewart CL, Schuchman EH: Acid sphingomyelinase deficient mice: a model of types A and B Niemann-Pick disease. *Nat Genet* 1995;10:288-293.
- 22 Ignarro RS, Vieira AS, Sartori CR, Langone F, Rogério F, Parada CA: JAK2 inhibition is neuroprotective and reduces astrogliosis after quinolinic acid striatal lesion in adult mice. *J Chem Neuroanat* 2013;48-49:14-22.
- 23 Kornhuber J, Tripal P, Reichel M, Terfloth L, Bleich S, Wiltfang J, Gulbins E: Identification of new functional inhibitors of acid sphingomyelinase using a structure-property-activity relation model. *J Med Chem* 2008;1:219-237.
- 24 Hurwitz R, Ferlinz K, Sandhoff K: The tricyclic antidepressant desipramine causes proteolytic degradation of lysosomal sphingomyelinase in human fibroblasts. *Biol Chem Hoppe Seyler* 1994;375:447-450.
- 25 Grassmé H, Jernigan PL, Hoehn RS, Wilker B, Soddemann M, Edwards MJ, Müller CP, Kornhuber J, Gulbins E: Inhibition of acid sphingomyelinase by antidepressants counteracts stress-induced activation of p38-kinase in major depression. *Neurosignals*, 2015;23:84-92.
- 26 Hoehn R, Monse M, Pohl E, Wranik S, Wilker B, Keitsch S, Soddemann M, Kornhuber J, Kohnen M, Edwards MJ, Grassmé H, Gulbins E: Melatonin acts as an antidepressant by inhibition of the acid sphingomyelinase/ceramide system. *Neurosignals* 2016;24:48-58.
- 27 Jernigan PL, Hoehn RS, Grassmé H, Edwards MJ, Müller CP, Kornhuber J, Gulbins E: Sphingolipids in major depression. *Neurosignals* 2015;23:49-58.
- 28 Kong E, Sucic S, Monje FJ, Reisinger SN, Savalli G, Diao W, Khan D, Ronovsky M, Cabatic M, Koban F, Freissmuth M, Pollak DD: STAT3 controls IL6-dependent regulation of serotonin transporter function and depression-like behavior. *Sci Rep* 2015;5:9009.
- 29 Tanaka Y: Recent progress and perspective in JAK inhibitors for rheumatoid arthritis: from bench to bedside. *J Biochem* 2015;158:173-179.