Hippocampal SIRT1 signaling mediates depression-like behavior

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ABSTRACT

BACKGROUND: Although depression is the leading cause of disability worldwide, its pathophysiology is poorly understood. Recent evidence has suggested that sirtuins (SIRT1) play a key role in cognition and synaptic plasticity, yet their role in mood regulation remains controversial. Here, we aimed to investigate whether SIRT1 function is associated with chronic stress-elicited depression-like behaviors and neuronal atrophy.

METHODS: We measured SIRT1 expression and activity in a mouse model of depression. We injected mice with a SIRT1 activator or inhibitor and measured their depression-like behaviors and dendritic spine morphology. To assess the role of SIRT1 directly, we used a viral-mediated gene transfer to overexpress the wild-type SIRT1 or dominant negative SIRT1 and evaluated their depression-like behaviors. Finally, we examined the role of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), a potential downstream target of SIRT1, in depression-like behavior.

RESULTS: We found that chronic stress reduced SIRT1 activity in the dentate gyrus of the hippocampus. Pharmacological and genetic inhibition of hippocampal SIRT1 function led to an increase in depression-like behaviors. Conversely, SIRT1 activation blocked both the development of depression-related phenotypes and aberrant dendritic structures elicited by chronic stress exposure. Furthermore, hippocampal SIRT1 activation increased the phosphorylation level of ERK1/2 in stressed condition, and viral-mediated activation and inhibition of hippocampal ERK2 led to anti- and pro-depressive behaviors, respectively.

CONCLUSION: Our results suggest that the hippocampal SIRT1 pathway contributes to the chronic stress-elicited depression-related phenotype and aberrant dendritic atrophy.

INTRODUCTION

Major depression is a highly prevalent disorder, and its symptoms include a depressed mood, loss of interest or pleasure, suicidal thoughts, reduced motivation, and hopelessness (1). Although the etiology and pathophysiology of this disease remain unclear, increasing evidence suggests that impaired neuronal plasticity contributes to one of the key underlying mechanisms (2-5). However, to date, the molecular mechanisms underlying altered neuronal plasticity associated with depression-like behavior remains to be fully determined.

Mammalian sirtuins (SIRT1-7) are deacetylase proteins that regulate diverse cellular processes, including aging, inflammation, and cellular stress resistance (6-8). SIRT1 acts on histones and other substrates in an NAD⁺-dependent manner (6, 9, 10). In the brain, SIRT1 has been reported to have many important functions related to circadian regulation (11-13), endocrine function (14), feeding (15-17), learning and memory, synaptic plasticity (18, 19), and reward (20, 21). Furthermore, SIRT1 dysfunction may be involved in the pathophysiology of neurodegenerative diseases and psychiatric disorders (22).

Recent studies have suggested that SIRT1 has important roles in the pathophysiology and treatment of depression. Genetic studies suggest that the *SIRT1* gene is associated with depressive and anxiety disorders (23-25). Other evidence has shown reduced levels of *SIRT1*, *SIRT2*, and *SIRT6* transcripts in patients with mood disorder (26). However, animal studies have produced conflicting results regarding the function of SIRT1 in depression and anxiety. Brain-specific SIRT1 knockout mice showed a reduced anxiety and stress-resilient phenotype, whereas global SIRT1 overexpression mice showed increased anxiety and stress vulnerability (24). In contrast,

another transgenic line overexpressing SIRT1 in the brain does not affect anxiety-like or depression-like behavior (27). In addition, although chronic stress exposure enhances SIRT1 activity in rats (28, 29), a SIRT activator has an antidepressant-like effect (30-32). Thus, SIRT-mediated control of depression and anxiety may depend on the cell type, genetic background, and brain region. However, the specific brain areas where SIRTs modulate these behaviors and their role in aberrant neuronal plasticity in depressed states remain largely unclear.

In this study, we sought to clarify the role of SIRTs in depression- and anxiety-like behavior as well as in neuronal plasticity. We first measured the mRNA level of SIRT1–7 in several brain areas of chronically stressed mice. Subsequently, we examined whether the inhibition or activation of hippocampal SIRT1 using pharmacological agents and an adeno-associated virus (AAV)-mediated gene transfer altered depression-like behaviors. Furthermore, we focused on extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) as the downstream targets of SIRT1 to explore the molecular pathways by which SIRT1 regulates depression-like behavior. Overall, our findings suggest that the hippocampal SIRT1 pathway mediates behavioral responses to chronic stress.

METHODS AND MATERIALS

A detailed description of the methods can be seen in Supplementary Information.

Animals

Adult male BALB/c (BALB) and C57BL/6J (B6) mice (Charles River Laboratories Japan) were maintained on a 12-h (light)/12-h (dark) cycle with *ad libitum* access to

mouse chow and water. Eight-week-old mice were used at the start of the experiments. All experimental procedures were performed according to the Guidelines for Animal Care and Use at Yamaguchi University Graduate School of Medicine.

Chronic ultra-mild stress (CUMS)

A CUMS procedure was conducted, as previously reported (33). This procedure was solely based on environmental and social stressors, which did not include food/water deprivation or any nociceptive events. Thus, this stress episode can be distinguished from unpredictable chronic mild stress (often referred to as UCMS or CMS). In brief, mice were subjected to a variety of stressors, such as cage tilt (30°), paired housing, confinement in small cages (11 × 8 × 8 cm), and soiled cages (50 mL water per liter of sawdust bedding). A reversed light/dark cycle was used from Friday evening to Monday morning. This procedure was scheduled over a 1-week period and repeated six times, but the reversed light/dark cycle was omitted during the weekend of the last week. Mice were subjected to a 1-h period of morning stress, a one 2-h period of afternoon stress, and overnight stress. Non-stressed (NS) mice were handled daily for weighing purposes.

Repeated restraint stress (RRS)

An RRS procedure was conducted, as previously reported (34) with minor modifications. Mice were individually subjected to restraint stress (4 h per day) for 10 consecutive days by placing them into a 30 mL plastic tube (2.7 cm in diameter and 8.15 cm long).

Drug administration

Imipramine (IMI) was dissolved in tap water at a concentration of 160 mg/l (35, 36). Sirtinol (10 μ M), EX-527 (1 μ M), and SRT2104 (10 μ M) were continuously injected bilaterally into the hippocampus. Resveratrol (RSV, 100 mg/kg/day) was daily injected intraperitoneally.

RNA and protein analysis

Quantitative real-time PCR was performed, as previously reported (33, 34). Western blotting was performed, as previously reported (37, 38). Immunofluorescence was performed on the free-floating sections, and the images were acquired using a Keyence BZ-9000 fluorescence microscope (Keyence).

Behavioral procedures

Behavioral assays were performed during the light phase (09:00–14:00 h), as previously reported (33, 39).

Virus-mediated gene transfer

AAV vectors were dissolved in physiological saline (0.5 μ l) and injected bilaterally into the dentate gyrus (DG) area [–2.0 mm anteroposterior (AP), $\pm$ 1.5 mm mediolateral (ML), –2.0 mm dorsoventral (DV) from the bregma (40)] at a rate of 0.1 μ l/min.

Statistical analyses

Complete statistical data are provided in Table S1 in Supplement 1. Data analyses were performed using an appropriate analysis of variance (one-way, two-way, or one-way repeated measures ANOVA). Significant effects were evaluated with Tukey's post hoc

tests or Bonferroni corrections. Unpaired *t* tests were used for comparisons between two groups. In all cases, *p* values were two-tailed and comparisons were considered to be statistically significant at a *p* value of <0.05. Data are presented as the mean $\pm$ SEM.

RESULTS

Chronic stress reduces hippocampal SIRT1 expression

We first investigated whether chronic stress alters the mRNA expression of SIRT1–7 in the hippocampus, amygdala, and medial prefrontal cortex, which are implicated in depression and anxiety (1, 41-44). We used chronically stressed BALB mice as a mouse model of depression (33). BALB mice were subjected to a 6-week period of CUMS episodes with or without chronic IMI treatment during the last 3 weeks of CUMS (Figure 1A). Real-time PCR analyses showed that compared with NS control mice, the mRNA expression of SIRT1 was significantly reduced in the whole hippocampus (Figure 1B) and DG (Figure 1C), but not in the Ammon's horn (CA; Figure 1D), of stressed mice, and this reduction in the whole hippocampus was also observed in stressed mice treated with IMI (Figure 1B). In addition, CUMS episodes increased the mRNA expression of SIRT6 in the hippocampus (Figure S1A in Supplement 1) and SIRT1 and SIRT2 in the amygdala (Figure S1B in Supplement 1). CUMS did not significantly affect SIRT1–7 mRNA expression levels (Figure S1C in Supplement 1), SIRT1 protein levels (Figure S2A in Supplement 1), and SIRT1 activity (Figure S2B in Supplement 1) in the medial prefrontal cortex. Western blotting confirmed that the SIRT1 protein level was significantly decreased in the hippocampus of stressed mice, and this reduction was not prevented by IMI treatment (Figure 1E and F). Furthermore,

we found that the reduced activity of SIRT1 in the hippocampus of stressed mice was prevented by chronic IMI treatment (Figure 1G). In contrast to BALB mice, C57BL/6J (B6) mice, a resilient strain to CUMS episodes (33), had no significant changes in SIRT1 mRNA levels and SIRT1 activity in the hippocampus (Figure S3 in Supplement 1). We further examined whether different types of stressors could also reduce SIRT1 mRNA. We subjected BALB mice to repeated restraint stress (4 h per day for 10 days) and measured their depression-like behavior and DG SIRT1 activity (Figure 1H). We found that restrained mice showed reduced social interaction time (Figure 1I) and sucrose preference (Figure 1J) as well as lower SIRT1 activity (Figure 1K). These results suggest the potential contribution of chronic stress-elicited SIRT1 dysfunction in the DG to the depression-related behavior.

Pharmacological inhibition of hippocampal SIRTs leads to depression-like behavior

We next investigated the behavioral effects of sirtinol, a SIRT1/2 inhibitor. We infused the DG of BALB mice bilaterally with sirtinol using osmotic minipumps (Figure 2A and B) and performed the social interaction test, which uses decreased social interaction time as an index of depression- and anxiety-like phenotypes (39, 45, 46). We found a significantly lower social interaction time in mice injected with sirtinol than in the vehicle-injected controls (Figure 2C). In the forced swim test, which uses decreased immobility time as the index of antidepressant-like efficacy (47), sirtinol treatment significantly increased the immobility time (Figure 2D). The novelty-suppressed feeding test uses a longer latency period to begin eating as the index of anxious phenotype (35, 39). We found that sirtinol treatment increased the latency to feeding in

mice (Figure 2E). We also performed the sucrose preference test to investigate anhedonic state (diminished interest or pleasure), but sirtinol treatment did not affect the sucrose preference (Figure 2F). We also bilaterally injected EX-527, a potent and selective SIRT1 inhibitor (48), into the DG of BALB mice (Figure 2G and H) and measured their depression-like behavior. We found that mice given EX-527 show a reduced social interaction time (Figure 2I) and an increased latency to feed in the novelty-suppressed feeding test (Figure 2K). However, they exhibited normal behaviors in forced swim and sucrose preference tests (Figure 2J and L). These results suggest that the inhibition of SIRT1 activity in the hippocampus induces depression-like and anxiety-like behaviors.

Pharmacological activation of hippocampal SIRT1s leads to stress resilience

We next assessed the effects of RSV, a nonspecific SIRT activator, on depression-like behavior. We intraperitoneally injected NS BALB mice with RSV and performed behavioral assays (Figure S4A in Supplement 1). We found that RSV injection did not alter any behavioral parameters (Figure S4B–G in Supplement 1). Subsequently, we examined the effect of RSV injection on depression-like behaviors in BALB mice subjected to CUMS (Figure S5A in Supplement 1). We found that increased depression-like and anxiety-like behaviors in stressed mice were prevented by RSV treatment (Figure S5B–G in Supplement 1). We also bilaterally injected SRT2104, a selective SIRT1 activator, into the DG of BALB mice subjected to RRS episodes (Figure 2M and N). SRT2104 treatment blocks reduced social interaction and sucrose preference induced by RRS exposure (Figure 2O and P). These results suggest that the SIRT1 activation lead to stress resilience.

AAV-mediated inhibition of SIRT1 activity increases depression-like behavior

One of the major limitations of an intra-DG injection is that the spread of the drug within and beyond the hippocampus is unpredictable. In addition, although our pharmacological data suggests a potential contribution of SIRT1 to depression-like behavior, it is unclear whether both EX-527 and SRT2104 are specific for SIRT1 *in vivo*. Therefore, we investigated the direct role of DG SIRT1 in depression-related behavior. To this end, we overexpressed either wild-type SIRT1 (wtSIRT1) or dominant-negative mouse SIRT1 (SIRT1 H355Y; dnSIRT1) (49) together with GFP in the DG of the dorsal hippocampus of BALB mice using the AAV-mediated gene transfer system (Figure 3A–C). GFP expression was observed in the dorsal DG neurons, but not in the GFAP-positive astrocytes or in CA3 pyramidal neurons (Figure S6 in Supplement 1), thus indicating a specific gene delivery in the dorsal DG neurons. The experimental design is shown in Figure 3D. We found that dnSIRT1 overexpression significantly decreased the social interaction time (Figure 3E). In the forced swim test, the overexpression of dnSIRT1 enhanced the immobility time (Figure 3F) but did not affect the latency to immobility (Figure 3G). In the novelty-suppressed feeding test, the latency to feeding was significantly increased by dnSIRT1 overexpression (Figure 3H) with no difference in the feeding activity (Figure 3I). These data suggest that the inhibition of the SIRT1 function in the DG leads to depression-like behaviors.

AAV-mediated overexpression of SIRT1 leads to chronic stress resiliency

In contrast to dnSIRT1 overexpression, the mice that overexpressed wtSIRT1 did not show any behavioral alterations under NS conditions (Figure 3E–I). We then

investigated the behavioral effect of wtSIRT1 overexpression under stressed conditions. The BALB mice were injected with AAV-wtSIRT1; after 3 weeks, they were subjected to CUMS for 6 weeks (Figure 3J). We found that the social interaction time in stressed mice overexpressing wtSIRT1 was significantly greater than that in stressed mice overexpressing GFP (Figure 3K). The immobility time and the latency to feeding in stressed mice overexpressing wtSIRT1 were significantly lesser than those in the control mice (Figure 3L and M). Thus, wtSIRT1 overexpression decreases the depression-like and anxiety-like behaviors in a stressed condition.

Involvement of SIRT in hippocampal structural plasticity

It has been suggested that depression is regulated by dynamic alterations in synaptic and dendritic structure and function (1, 2, 43). Earlier studies demonstrated that chronic stress episodes and treatment with an antidepressant-modulated hippocampal dendritic remodeling in rodents (50-52). In fact, Golgi staining revealed that CUMS induced neuronal atrophy and spine loss in the DG granule neurons, and that these effects were blocked by chronic treatment with IMI (Figure 4A–E). We then examined whether SIRT inhibition leads to aberrant dendritic structures, as observed in stressed mice. The mice were continuously infused bilaterally with sirtinol in the hippocampus via osmotic minipumps (Figure 4F). We found reduced dendritic length and spine loss in the granule neuron dendrites of mice receiving sirtinol (Figure 4G–J). Conversely, systemic administration of RSV blocked the chronic stress-elicited aberrant dendritic structures (Figure S7 in Supplement 1). Although there were no significant effects of intra-hippocampal infusion of SRT2104 on neuronal morphology in NS condition (Figure S8 in Supplement), this drug significantly enhanced spine loss, but no effect on

dendritic length, in stressed condition (Figure 4M–O). These results indicate that the SIRT1 function is involved in structural remodeling induced by chronic stress episodes.

SIRT1 regulates neurite outgrowth

To confirm the role of SIRT1 in neurite outgrowth, we initially used Neuro2a cells, which are commonly used model system, in combination with hippocampal neurons to study the pathway involved in neuronal morphology (53-56). We found that the percentage of neurite-bearing cells was increased by treatment with 5 μ M RSV for 48 h (Figure 5A and B). When Neuro2a cells grown under low serum conditions, which promotes neurite outgrowth, were treated with 1, 5, or 10 μ M sirtinol for 48 h, the percentage of neurite-bearing cells had decreased dramatically compared with the vehicle controls (Figure 5C and D). Thus, SIRT modulators can lead to altered neurite outgrowth in cultures. Next, we examined whether the neurite-promoting effect of RSV would result from the activation of SIRT1. To investigate this possibility, we treated RSV (5 or 10 μ M) to Neuro2a cells transfected with siRNA against SIRT1 to knockdown the expression of SIRT1. Western blotting indicated that the siRNA successfully suppressed SIRT1 in Neuro2a cells (Figure 5E). We found that SIRT1 knockdown blocked the percentage increase of neurite-bearing cells induced by treatment with 5 μ M RSV, whereas there was no effect of the SIRT1 siRNA when treated with 10 μ M RSV (Figure 5F). We investigated the effect of overexpression of either wtSIRT1 or dnSIRT1 on neurite outgrowth (Figure 5G and H). We found that wtSIRT1 overexpression led to an increase in the percentage of neurite-bearing cells, but there was no effect of dnSIRT1 overexpression on neurite outgrowth (Figure 5H).

Subsequently, we determined whether SIRT1 could influence dendritic morphology in primary mouse hippocampal neurons. We found that the overexpression of wtSIRT1, but not dnSIRT1, enhanced the number of dendritic intersections (Figure 5I and J). In addition, the treatment of primary hippocampal neurons with 0.5 μ M SRT1720, a SIRT1 activator, increased the number of dendritic intersections compared with the vehicle treatment (Figure 5K and L). Together, these cell culture experiments suggest that SIRT1 promotes neurite outgrowth.

Role of ERK1/2, a potential downstream target of SIRT1, in behavioral responses to chronic stress

We investigated the molecular pathway(s) underlying the SIRT1-mediated behavioral responses to stress. SIRT1 is required for the normal activation of ERK1/2 (57), proteins known to be associated with depression and antidepressant action in humans and rodents (58-61) as well as with neuronal plasticity (62-64). These facts led us to speculate that chronic stress-mediated downregulation of SIRT1 activity could affect ERK1/2 function, thereby modulating neural plasticity and subsequent behavioral responses to stress. This hypothesis is supported, in part, by our findings that CUMS reduced the level of phosphorylated ERK1/2 (pERK1/2) in the hippocampus, and that this reduction was blocked by continuous treatment with IMI (Figure 6A). In addition, we found that a continuous infusion of sirtinol into the hippocampus reduced pERK1/2 levels in the DG (Figure S9 in Supplement). Furthermore, we found that a continuous infusion of SRT2104 into the hippocampus blocked a RRS-induced reduction of pERK1/2 in the DG (Figure 6B). To directly investigate the role of hippocampal ERK1/2 on behavior, we overexpressed wtERK2, dominant-negative ERK2

(ERK2-K52R, dnERK2), or GFP in the DG of BALB mice using an AAV-mediated gene transfer (Figure 6C–F). We found no significant effects from the overexpression of either wtERK2 or dnERK2 on social interaction and sucrose preference tests (Figure 6G and J). However, wtERK2 overexpression significantly reduced the immobility time in the forced swim test (Figure 6H) and the latency to feeding in the novelty-suppressed feeding test (Figure 6I). Conversely, the overexpression of dnERK2 enhanced the immobility time (Figure 6H) and the latency to feeding (Figure 6I). These results suggest that hippocampal ERK2 function is involved in anxiety- and depression-like behaviors.

DISCUSSION

Our results demonstrate that altering the expression and function of SIRT1 in the DG mediates depression-like behavior. We found that increasing hippocampal SIRT1 function blocks depression-like behavior caused by chronic stress, resulting in a state of stress resilience. Conversely, the inhibition of the SIRT1 function drives prodepressive behavior. In addition, we found that chronic stress-elicited spine loss was prevented by SRT2104 treatment, whereas sirtinol treatment induced aberrant dendritic remodeling. Together with the results from the primary hippocampal neurons, chronic stress-elicited aberrant dendritic structures may be caused by a SIRT1 dysfunction. Moreover, we found that SIRT1 activation prevented a chronic stress-elicited downregulation of pERK1/2, and that gain- and loss-of-function ERK2 led to stress resilience or prodepressive behaviors, respectively. Thus, our findings suggest that a dysfunction of hippocampal SIRT1 signaling results in aberrant structural plasticity and depression-like behavior.

In contrast to our results, a recent study indicated that brain-specific SIRT1 knockout mice reduced anxiety and developed resilience to social defeat-induced depression, whereas global SIRT1 overexpression mice had increased anxiety and showed a susceptibility to depression (24). The reason for the discrepancy between this and our study is unclear, but one possibility may be the use of mice with different genetic backgrounds. Brain-specific SIRT1 knockout and SIRT1-overexpressing mice used by Libert et al. (24) were generated by backcrossing SIRT1-floxed mice (mixed genetic background) or SIRT1-knockin mice (mixed genetic background) with C57BL/6 mice (65, 66). It should be noted that even after 12 generations of backcrossing, the chromosome segment containing genes from the ES-cell donor strain could contain as much as 1% of the genome, and there is still a chance that flanking genes influence behavioral outcomes (67, 68). Animals with distinct genetic backgrounds exhibit substantial differences in gene expression and stress reactivity, and these animals can be used as models for investigating the influence of genetic and environmental factors on brain function and behaviors (33, 69-71). Indeed, brain-/spinal cord-specific SIRT1-overexpressing mice (C57BL/6J background) did not show obvious behavioral deficits in depression- and anxiety-like behaviors (27). In addition, we found that there was no effect of CUMS exposure on SIRT1 expression in C57BL/6J mice. Depression is a heterogeneous disorder resulting from the interaction of multiple genetic factors with environmental factors (1). Thus, differences between the regulation of SIRT1 expression and function because of stress among genetic backgrounds and among species may support the complex mechanisms underlying the etiology of depression. Future research will be required to clarify the mechanisms of how the genetic-environment interaction influences SIRT1 expression and activity and to clarify

its role in depression.

Other studies showed that chronic stress exposure enhances SIRT1 activity in the DG of rats (28, 29) in direct contradiction to our findings. It is important to note that SIRT1 expression has a circadian rhythm (12). Therefore, it is possible that SIRT1 expression and activity have a shift in the rhythm due to the effect of chronic stress exposure and may depend on time of the day tissues are collected. In the further studies, tissues samples need to be collected at various time points to test this correlation.

Another possible explanation for the discrepancy is that the role of SIRT1 in modulating mood and anxiety is tissue- and cell-type specific. Our study showed opposite effect of CUMS on SIRT1 mRNA expression between the DG and amygdala. In addition, it has been suggested that the dorsal subregion of the hippocampus has a preferential role in memory, whereas the ventral subdivision is involved in anxiety and stress responses (72, 73). Although our study with AAV vectors provides new insight into the dorsal DG-specific role of SIRT1 in behavioral responses to chronic stress, we did not demonstrate the specific role of SIRT1 in the amygdala and ventral hippocampus in depression and anxiety. Further experiments are needed to address this issue.

SIRT1 has been shown to stimulate neurite development in primary hippocampal neurons (74, 75) and cell lines (76-78), and this is in agreement with our present data. It is important to note that SIRT1 loss-of-function mice had reduced dendritic complexity, shorter branches, and spine loss (18, 19). Together with these *in vitro* and *in vivo* data, our results further suggest that SIRT1 plays a key role in chronic stress-elicited aberrant neuronal plasticity. However, our study has a technical limitation. We found that treatment with SRT1720 increased the number of dendritic intersections

in the primary culture of hippocampal neurons, whereas there were no effects of SIRT1 activators on dendritic structures in NS mice as assessed by Golgi staining. This discrepancy could be because of the differences in methodology (Golgi staining and fluorescent labeling) and biological systems (brain and cell culture). Golgi staining does not always reliably label all dendritic/axonal branches and fine terminal arborizations, and this limitation may affect the results in this study.

Given that SIRT1 inhibition reduces ERK1/2 activity, partly through the inactivation of IRS-2, and ERK1/2 phosphorylation is decreased in the hippocampus of SIRT1-knockout mice (57), we focused on ERK1/2 as potential target molecules for SIRT1 in a second set of experiments. Indeed, we and other groups have indicated that SIRT1 activators and inhibitors enhance or reduce ERK1/2 phosphorylation, respectively, in the brains (21, 30). Although there is a potential link between SIRT1 and ERK1/2 in the modulation of depression-like behaviors, we did not demonstrate a direct contribution of the SIRT1-ERK1/2 pathway in chronic-stress-elicited depression-like behavior. One of the major roles of SIRT1 is to control gene expression by modulating the deacetylation of histones and transcription factors (79). Recent evidence demonstrated that SIRT1 directly deacetylated the transcription factor heat shock factor 1 (HSF1), which increased the gene transcription of molecular chaperones that help maintain intracellular protein homeostasis (80). Interestingly, hippocampal HSF1 has been reported to be involved in the structural plasticity of dendritic granule neurons as well as in the behavioral response to chronic stress (39). This suggests that SIRT1-HSF1 signaling is another potential direct pathway for controlling neuronal plasticity and behavioral responses to stress. Thus, SIRTs may have diverse roles in controlling gene expression by stimulating intracellular signaling pathways (e.g.,

IRS-2-ERK1/2) and by deacetylating transcription factors (e.g., HSF1) and histones epigenetically (22). Future studies will be necessary to completely elucidate the role of the downstream signaling pathways of SIRT1 in the regulation of mood and anxiety.

In conclusion, we describe one of the molecular mechanisms by which chronic stress negatively affects structural plasticity and behavior. The results indicate that SIRT1 in the DG mediates behavioral responses to chronic stress as well as to chronic stress-elicited dendritic atrophy. Our preclinical evidence supports aberrant SIRT1 function as a potential molecular mechanism underlying depression and further suggests that agents stimulating SIRT1 signaling represent a promising new therapeutic approach to depression.

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FIGURE LEGENDS

Figure 1. Chronic stress reduces SIRT1 expression in the hippocampus.

A. Schematic of the experimental design. BALB mice were subjected to chronic ultra-mild stress (CUMS) for 6 weeks. The antidepressant IMI was administered for the last 3 weeks of the CUMS session. Stressed and NS mice receiving IMI or water, respectively, were then sacrificed the day after the last CUMS session.

B. mRNA expression of SIRT1 in the whole hippocampus of the mice subjected to CUMS or NS conditions with or without continuous IMI treatment (n = 6 per group). * $p < 0.05$ vs. NS mice receiving tap water.

C and D. Levels of SIRT1 mRNA in the DG (**C**) and CA (**D**) in the mice subjected to CUMS or the NS condition (n = 6 per group). * $p < 0.05$.

E and F. Levels of SIRT1 protein in the hippocampus of the mice subjected to CUMS or the NS condition with or without continuous IMI treatment (n = 7-8 per group). * $p < 0.05$ vs. NS mice receiving tap water.

G. Quantification of SIRT1 activity in the DG of the hippocampus of stressed mice, with or without continuous IMI treatment (n = 8 per group). * $p < 0.05$ vs. NS mice receiving tap water.

H. Schematic of the experimental design. BALB mice were subjected to repeated restraint stress (RRS; 4 h per day) for 10 days. The mice were then tested using behavioral assays. SI, social interaction test; SPT, sucrose preference test.

I and J. Behavioral data are shown. (**I**) is the interaction time in the social interaction test and (**J**) is sucrose preference in the sucrose preference test (n = 12 per group). * $p < 0.05$.

K. Quantification of SIRT1 activity in the DG of the hippocampus of mice subjected to

RRS (n = 6 per group). * $p < 0.05$. Data are presented as the mean $\pm$ SEM.

Figure 2. Pharmacological manipulation of SIRT activity alters depression-like behavior.

A. Schematic of the experimental design. BALB mice were implanted with osmotic minipumps filled with sirtinol or a vehicle and using guided cannulae directed to the hippocampus. Two weeks after the surgery, the mice were tested using behavioral assays. SI, social interaction test; FST, forced swim test; NSF, novelty-suppressed feeding test; SPT, sucrose preference test.

B. The locations of the cannula tips are shown.

C–F. Behavioral data are shown. (**C**) is the social interaction time, (**D**) is the immobility time in the forced swim test, (**E**) is the latency to feeding in the novelty-suppressed feeding test, and (**F**) is the sucrose preference in the sucrose preference test (n = 13–14 per group). * $p < 0.05$.

G. Schematic of the experimental design. BALB mice were bilaterally injected with EX-527 or vehicle into the hippocampus using osmotic minipumps. Two weeks after the surgery, the mice were tested using behavioral assays.

H. The locations of the cannula tips.

I–L. Behavioral data are shown. (**I**) is the social interaction time, (**J**) is the immobility time in the forced swim test, (**K**) is the latency to feeding in the novelty-suppressed feeding test, and (**L**) is sucrose preference in the sucrose preference test (n = 12–13 per group). * $p < 0.05$.

M. Schematic of the experimental design. BALB mice were injected bilaterally with SRT2104 or vehicle into the hippocampus using osmotic minipumps. One week after the surgery, mice were subjected to repeated restraint stress (RRS; 4 h per day) for 10

days. The mice were then tested using behavioral assays.

N. The locations of the cannula tips.

O and **P.** Behavioral data are shown. (**O**) is the social interaction time and (**P**) is sucrose preference in the sucrose preference test (n = 12–14 per group). * $p < 0.05$ vs. NS mice receiving vehicle.

Data are presented as the mean $\pm$ SEM.

Figure 3. SIRT1 is required for behavioral response to chronic stress.

A. Schematics of the AAV vectors engineered to overexpress wild-type SIRT1 (wtSIRT1), dominant negative SIRT1 (SIRT1-H355Y, dnSIRT1), or GFP.

B and **C.** Successful transductions of GFP, wtSIRT and dnSIRT1 using the AAV-mediated gene transfer. (**B**) Left, coronal brain images adapted from Paxinos and Franklin, (2001). Right, corresponding image showing transgene expression (GFP, green) following vector microinjection (counterstained with DAPI, blue). (**C**) Western blotting shows overexpression of wtSIRT1 and dnSIRT1 in the hippocampus.

D. Schematic of the experimental design. BALB mice were injected bilaterally with AAV into the DG, and after 3 weeks, they were tested in the behavioral assays. SI, social interaction test; FST, forced swim test; NSF, novelty-suppressed feeding test.

E–I. Behavioral data are shown; (**E**) is the social interaction time, (**F**) is the immobility time, (**G**) is the latency to immobility in the forced swim test, (**H**) is the latency to feeding, and (**I**) is the body weight loss in the novelty-suppressed feeding test (n = 13–14 per group). * $p < 0.05$ vs. mice given AAV-GFP.

J. Schematic of the experimental design. BALB mice were injected bilaterally with AAV-wtSIRT1 or AAV-GFP in the DG. After 3 weeks, they were subjected to CUMS

for 6 weeks and then tested in the behavioral assays.

K–M. Behavioral data are shown; (**K**) is the social interaction time, (**L**) is the immobility time in the forced swim test, and (**M**) is the latency to feeding in the novelty-suppressed feeding test (n = 14–15 per group). * $p < 0.05$. The data are presented as the mean $\pm$ SEM.

Figure 4. SIRT activity regulates chronic stress-elicited structural plasticity.

A. Schematic of the experimental design. BALB mice were subjected to CUMS for 6 weeks. The antidepressant IMI was administered for the last 3 weeks of the CUMS session.

B and C. Representative images of DG granule neurons in the mice subjected to CUMS with or without IMI treatment. The bar graph shows the total dendrite length of DG granule neurons; n = 5–6 per group. * $p < 0.05$ vs. NS. Scale bars, 50 μm .

D and E. Representative images of dendritic spines from DG granule neurons in the mice subjected to CUMS with or without IMI treatment. The bar graph shows the spine densities of DG granule neurons; n = 5–6 per group. * $p < 0.05$ vs. NS. Scale bars, 10 μm .

F. Schematic of the experimental design. BALB mice were implanted with osmotic minipumps filled with sirtinol or a vehicle, and a guided cannulae targeted the hippocampus. Two weeks after the surgery, the mice were sacrificed for Golgi staining.

G and H. Representative images of DG granule neurons in mice injected with sirtinol or a vehicle. The bar graph shows the total dendrite length of DG granule neurons; n = 6 per group. * $p < 0.05$. Scale bars, 50 μm .

I and **J**. Representative images of dendritic spines from DG granule neurons in mice treated with sirtiol or a vehicle. The bar graph shows the spine densities of DG granule neurons. $n = 5-6$ per group. $*p < 0.05$. Scale bars, 10 μm .

K. Schematic of the experimental design. BALB mice were subjected to a 10-day RRS session (4 h per day) and were bilaterally injected with SRT2104 or a vehicle.

L and **M**. Representative images of DG granule neurons in stressed mice injected with SRT2104 or a vehicle. The bar graph shows the total dendrite length of DG granule neurons ($n = 5-6$ per group).

N and **O**. Representative images of dendritic spines from DG granule neurons in stressed mice treated with SRT2104 or a vehicle. The bar graph shows the spine densities of DG granule neurons ($n = 5-6$ per group). $*p < 0.05$.

Data are presented as the mean $\pm$ SEM.

Figure 5. SIRT1 controls neurite outgrowth in cultured cells.

A and **B**. Treatment of Neuro2a cells with 5 μM RSV results in an increased percentage of neurite-bearing cells. $*p < 0.05$ vs. vehicle.

C and **D**. Treatment of Neuro2a cells with sirtinol (1, 5, or 10 μM) results in a decreased percentage of neurite-bearing cells in the serum-starvation condition. $*p < 0.05$ vs. vehicle.

E and **F**. Effect of SIRT1 knockdown by siRNA on RSV-induced enhancement of neurite outgrowth. Western blotting shows the successful knockdown of SIRT1 protein by the siSIRT1 transfection (**E**). The bar graph shows the percent of neurite-bearing cells (**F**). $*p < 0.05$ vs. vehicle.

G and **H**. Effect of wtSIRT1 or dnSIRT1 overexpression on neurite outgrowth. Western blotting shows the successful overexpression of wtSIRT1 or dnSIRT1 protein (**G**). The bar graph shows the percentage of neurite-bearing cells (**H**). $*p < 0.05$ vs. control.

I and **J**. Sholl analysis shows the number of intersections in primary mouse hippocampal neurons transfected with GFP and wtSIRT1 or dnSIRT1. Scale bar, 100 μm . $*p < 0.05$ vs. control.

K and **L**. Sholl analysis shows the number of intersections in primary mouse hippocampal neurons treated with SRT1720 (0.1 or 0.5 μM) or a vehicle. Scale bar, 100 μm . $*p < 0.05$ vs. control.

Data are presented as the mean $\pm$ SEM.

Figure 6. Hippocampal ERK1/2 function is associated with behavioral responses to chronic stress.

A. Western blot analyses of the pERK1/2 and total ERK1/2 protein levels in the hippocampus of the mice subjected to CUMS or NS conditions with or without IMI treatment. $n = 7-8$ per group. $*p < 0.05$ vs. NS mice receiving tap water.

B. Western blot analyses of the pERK1/2 and total ERK1/2 protein levels in the hippocampus of mice subjected to RRS (4 h per day for 10 days) or NS conditions with or without an intra-hippocampal SRT2104 injection ($n = 6$ per group). $*p < 0.05$ vs. NS mice receiving a vehicle.

C. Schematics of the AAV vectors engineered to overexpress wild-type ERK2 (wtERK2), dominant-negative ERK2 (ERK2-K52R, dnERK2), or GFP under the control of the CMV promoter.

D and **E**. Successful transductions of GFP, wtERK2, and dnERK2 using the AAV-mediated gene transfer.

F. Schematic of the experimental design. BALB mice were injected bilaterally with AAV in the DG, and after 3 weeks, they were tested in the behavioral assays. SI, social interaction test; FST, forced swim test; NSF, novelty-suppressed feeding test; SPT, sucrose preference test.

G-J. Behavioral data are shown. (**G**) is the social interaction time, (**H**) is the immobility time in the forced swim test, (**I**) is the latency to feeding in the novelty-suppressed feeding test, and (**J**) is the sucrose preference in the sucrose preference test (n = 12–14 per group). * $p < 0.05$ vs. mice given AAV-GFP.

Data are presented as the mean $\pm$ SEM.

Figure 1

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