Lithium Down-regulates Histone Deacetylase 1 (HDAC1) and Induces Degradation of Mutant Huntingtin*

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Background: Lithium and valproic acid (VPA) exhibit a robust neuroprotective and anti-tumor effects in neural cells and tumor cells.

Results: Lithium significantly down-regulates HDAC1 at the translational level by targeting HDAC1 mRNA.

Conclusion: The decrease in HDAC1 is essential for the lithium-mediated, autophagic degradation of mutant huntingtin.

Significance: This report is the first demonstration that HDAC1 decreases in response to lithium treatment.

Lithium is an effective mood stabilizer that has been clinically used to treat bipolar disorder for several decades. Recent studies have suggested that lithium possesses robust neuroprotective and anti-tumor properties. Thus far, a large number of lithium targets have been discovered. Here, we report for the first time that HDAC1 is a target of lithium. Lithium significantly down-regulated HDAC1 at the translational level by targeting HDAC1 mRNA. We also showed that depletion of HDAC1 is essential for the neuroprotective effects of lithium and for the lithium-mediated degradation of mutant huntingtin through the autophagic pathway. Our studies explain the multiple functions of lithium and reveal a novel mechanism for the function of lithium in neurodegeneration.

Lithium was revealed as the first efficacious mood stabilizer for the treatment of bipolar disorder in 1950. Together with valproic acid (VPA), a histone deacetylases inhibitor, lithium remains the most commonly used drug for mental diseases (1, 2). Accumulating evidence indicates that lithium also plays a neuroprotective role in neurodegenerative diseases such as Alzheimer disease (3–5), Parkinson disease (6–8), and Huntington disease (9, 10). Moreover, lithium has been found to be a multifunctional ion in a variety of cellular processes including cell proliferation (11), the cell cycle, and cell apoptosis (12, 13) as well as embryonic development (14) and tissue formation (15). However, the exact molecular mechanisms by which lithium functions in these biological processes remain to be elucidated.

The main target of lithium is glycogen synthase kinase 3β (GSK3β) (16). Lithium can directly and indirectly inhibit GSK3β activity by acting as a competitive inhibitor of Mg2+ and inhibiting the dephosphorylation of GSK3β (17); lithium further regulates a multitude of GSK3β downstream signaling pathways, such as glycogen metabolism, gene transcription, and protein synthesis (18). Studies suggest that several GSK3β target genes have roles in neurodegenerative diseases and tumorigenesis (19–22). Thus, the inhibitory effect of lithium on GSK3β partially explains its functional diversity.

In vitro studies have demonstrated that lithium can induce autophagy, which is a major intracellular degradation system that utilizes lysosomes to degrade long-lived proteins and damaged organelles (23, 24). As a crucial recycling system, autophagy is essential for intracellular quality control, cell death and differentiation (25, 26), tumor suppression (27, 28), organism development, and other processes. Defects in autophagy have been frequently associated with diseases as well as tumorigenesis, immune deficiency, and neurodegeneration (29, 30). Lithium-induced autophagy promotes the clearance of toxic, long-lived, aggregate-prone proteins, such as mutant huntingtin, α-synuclein (8), and even pathological prions (31). This evidence partially explains the neuroprotective effects of lithium in neurodegenerative diseases.

Histone acetylation and deacetylation regulate the remodeling of chromatin structure and influence gene expression. Abnormal expression of histone deacetylases (HDACs) has always been related to tumor formation and other pathological conditions, such as neurodegeneration (32–36). Huntington disease is a neurodegenerative disorder caused by the polyglutamine repeat in the N terminus of the huntingtin protein (37). Mutant huntingtin, which contains a fragment of 35 repeated glutamines, tends to accumulate in inclusion bodies in neural
cells and causes severe motor and cognitive impairments. Degradation of mutant huntingtin through autophagy can decrease the toxicity of these aggregates. Jeong et al. (38) reported that the HDAC1-mediated deacetylation of mutant huntingtin enhances its stability and prevents its degradation through autophagy; however, overexpression of the histone acetylase cAMP-response element-binding protein (CREB)-binding protein (CBP) accelerates the degradation of mutant huntingtin. This process may contribute to the loss of acetylation homeostasis in neurodegenerative conditions (33, 39–41).

Lithium and VPA have been shown to be effective treatments for neurodegenerative diseases; however, the underlying mechanism is not fully understood. In this study we showed that lithium decreases HDAC1 protein levels by inhibiting the translation of HDAC1, which is required for the lysosomal degradation of mutant huntingtin. Our experimental evidence indicates that HDAC1 might be a novel therapeutic target for neural diseases, such as bipolar disorder.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—The HDAC1 promoter, HDAC1 3′-untranslated region (3′-UTR), and p21 promoter were cloned and inserted into pG3L3-basic (Promega Corp.). The HDAC1 gene was cloned and inserted into the modified plVX-IREs-puromycin plasmid (Clontech Laboratories, Inc.). Htt590-100Q was generated from the normal Htt590–23Q construct, which was cloned from HEK293T cDNA; in this construct, the 23Q was replaced with 100Q from the Htt171-100Q construct provided by Hongyu Hu (Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences) (42). The CUGBP1 and Eif-2β genes were obtained from Jiahui Han (Xiamen University).

**Cell Transfection and Lentiviral Infection**—HEK 293T and HeLa cells were transfected using Effectene (Qiagen) according to the manufacturer’s protocol. For stable transfection, HeLa cells were infected with lentiviruses expressing HDAC1-FLAG, Htt590-100Q-His, or Htt590-100Q (K444R). Cells stably expressing the constructs were selected by incubation with 1–2 μg/ml puromycin beginning at 48 h post-infection.

**Chemical Inhibitors**—MG-132, benzoxycarbonyl-VAD, cycloheximide, leptomycin B, and sodium butyrate were purchased from Beyotime. Lactacystin, 3-methyladenine, SB-216763, cycloheximide, leptomycin B, and sodium butyrate were purchased from Selleck. VPA was purchased from Merck.

**RT-PCR and Quantitative PCR Analyses**—For RT-PCR, total RNA was isolated using the TRIzol reagent (Invitrogen) and was used for RT-PCR with the ReverTra Ace qPCR RT kit (TOYOBO) or the SYBR® PrimeScript® miRNA RT-PCR kit (Takara). For miRNA RT-PCR, the following RT primers specific for miR-449a and 5 S rRNA were used: RT primer for miR-449a, 5′-CTCACTTGTTGTCTGGAGTGCGGCATTCA-GTGGAGGACCAGCTA-3′; RT primer for 5 S rRNA, 5′-CTCACTTGTTGTCTGGAGTGCGGCATTCA-GTGGAGGACCAGCTA-3′.

**qPCR analysis** was performed using the 7500-Fast Real-Time PCR Systems with the supplied software (Applied Biosystems) and the following primers: HDAC1 forward (5′-TAAAATTCTTGCGCTCCATCC-3′) and reverse (5′-AACAGGGCCATCG-AATCTGG-3′); HDAC2 forward (5′-GCTGCGCCATCTT-GAATTACTAA-3′) and reverse (5′-TTATGACTCATCATCTATACCATCT-3′); CUGBP1 forward (5′-ATGGCAGACAGGCTATCAAGG-3′) and reverse (5′-CAACAGATGCCTGCGCTGATTTGC-3′); EIF2A forward (5′-GCTTCTTCTGTCTCAGTCAAGC-3′) and reverse (5′-CTCTCCATCTGGTCCTCAAGTT-3′); p21 forward (5′-AGGTTGAGACCTTGAGACTCTCAG-3′) and reverse (5′-TCTCTTGGAGAGATCACGG-3′); β-catenin forward (5′-CACAAGCAGAGTGCAGAGTTG-3′) and reverse (5′-GATTCCCTGAGAGTTCAAAGACAC-3′); DKK-1 forward (5′-GGATATTCCAGAAGACACCCTTG-3′) and reverse (5′-CTTGGAGGAGACTTGCTCAGC-3′); p21 forward (5′-AGGTTGAGACCTTGAGACTCTCAG-3′) and reverse (5′-TCTCTTGGAGAGATCACGG-3′); GAPDH forward (5′-GTCTTCTCTGTACTCAACGG-3′) and reverse (5′-ACCACCCCTTGCTGATGACAC-3′); miR-449a forward (5′-TGCGTTGCAGTGATTTTGCTGAGTGGT-3′) and reverse (5′-CTCAACTTGTTGCTGGAGTCCGGA-3′); 5 S rRNA forward (5′-CTGCGCGCTGGGAAATACCGGT-GTGCAGTGGAGGAGACTCTCAG-3′) and reverse (5′-CTCAACTTGTTGCTGGGAAATACCGGT-GTGCAGTGGAGGAGACTCTCAG-3′).

**Western Blot Analysis and Co-immunoprecipitation**—Whole cell lysates were prepared using radioimmune precipitation assay buffer or immunoprecipitation lysis buffer (Beyotime). The supernatants were size-fractionated by 10% SDS-PAGE. The blots were incubated with primary antibodies recognizing actin (I-19, Santa Cruz), GAPDH (cwbio), β-catenin (E-5, Santa Cruz), HDAC1 (C-19, Santa Cruz), HDAC2 (Beyotime), histone H3 (FL-136, Santa Cruz), FLAG tag (3B9, Abmart), His tag antibody and p62 antibody, respectively.

**Immunofluorescence Staining**—The cells were fixed with 4% formaldehyde, permeabilized with 0.5% Triton X-100, and incubated in 5% BSA for 1 h. The samples were incubated with the primary antibody overnight at 4 °C and subsequently incubated with a secondary antibody conjugated to Alexa Fluor-595 (Molecular Probes) for 1 h at room temperature. Images were processed using a Leica SP2 microscope equipped with a 40× objective.
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**RESULTS**

**Lithium Down-regulates HDAC1 in a Time- and Dose-dependent Manner**—The similar clinical efficacy of lithium and VPA allowed us to explore the relationship between lithium and cellular acetylation. We first investigated whether HDAC1 is a target of lithium. HEK293T, SW480, and HeLa cells were treated with lithium for various time periods. As shown in Fig. 1, A–C, HDAC1 protein levels were decreased in all three cell lines after the addition of lithium. Additionally, HDAC1 protein levels were diminished in a lithium dose-dependent manner in HeLa cells (Fig. 1D). Cell lysate fractionation was performed using the SW480 cells to determine the subcellular localization of HDAC1. We found that the level of

**HDAC Activity Assay**—HDAC activity was measured using the Colorimetric HDAC Activity Assay kit (K331-100, BioVision) according to the manufacturer’s protocol. HDAC colorimetric substrate was incubated with 150 μg of HeLa cell lysate, and the signal was read at 405 nm. HDAC activity is expressed as the relative absorbance value per μg of protein.

**Luciferase Reporter Assay**—The pGL3-HDAC1 promoter, pGL3-HDAC1–3′-UTR, pGL3-p21 promoter, or pGL3-mini-p21 promoter plasmid and the Renilla luciferase plasmid (Promega) were co-transfected into HEK 293T cells. Lithium was added 18 h after transfection. The firefly and Renilla luciferase activities were measured 24 h after treatment using the Dual-Glo Luciferase Assay System (Promega).

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**FIGURE 1.** HDAC1 is specifically down-regulated by lithium in a time- and dose-dependent manner. A–C, HDAC1 was down-regulated after lithium treatment in different cell lines. HEK293T (A), HeLa (B), and SW480 (C) cells were treated with 20, 40, and 20 mM LiCl, respectively, for the indicated times. β-Catenin was used as a positive control to confirm the efficacy of LiCl. D, HDAC1 was down-regulated by LiCl in a dose-dependent manner. HeLa cells were treated with different concentrations of LiCl for 24 h. E, SW480 cells were treated with 20 mM LiCl and then fractionated into nuclear and cytoplasmic extracts. F, HDAC2 was not regulated by lithium. HeLa cells were treated with 20 mM LiCl for the indicated times. HDAC2 levels were detected by Western blot analysis. G, NaCl had no effect on HDAC1 protein levels. 293T cells were treated with the indicated concentrations of NaCl for 24 h. H, HeLa and SW480 cells were treated with LiCl for the indicated times. p21 levels were measured by qPCR and were normalized to GAPDH expression. J, schematic diagram of the transcription factor binding sites in the p21 promoter and mini-p21 promoter. J, HEK293T cells were treated with 20 mM LiCl for 18 h after transfection with the p21 promoter reporter plasmid or the mini-p21 promoter reporter plasmid and pCDNA3-HDAC1. Luciferase activity was measured after treatment with LiCl for 24 h. EV, empty vector. K, HDAC activity was measured using 150 μg of HeLa cell lysate treated with 30 mM LiCl for the indicated times. HeLa lysate treated with 0.5 mM VPA was used as a control. **, p < 0.01; ***, p < 0.001.
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HDAC1 in the nuclear extracts of SW480 cells decreased over time (Fig. 1E). Interestingly, another class I HDAC, HDAC2, which shares up to 80% overall sequence identity with HDAC1, was not affected by lithium in the HeLa cell line (Fig. 1F). To exclude the possibility that chlorine ions induce these effects, sodium chloride was used to treat 293T cells and was found to have no effect on HDAC1 protein levels (Fig. 1G). Taken together, these data indicate that HDAC1 was decreased specifically by lithium.

Next, we sought to determine whether lithium treatment can derepress the downstream target genes of HDAC1. p21 is a classic target gene of HDAC1 (43, 44), and HDAC1 represses p21 transcription by associating with the transcription factor SP1 on the p21 promoter (45, 46). We found that lithium significantly up-regulated p21 mRNA levels based on qPCR analysis (Fig. 1H). To further validate this result, we performed a p21 luciferase reporter assay. The results showed that p21 promoter activity was obviously increased in the presence of lithium (Fig. 1I). Next, we analyzed potential transcription factor binding sites in the p21 promoter using an online analysis system (CONSITE). In addition to the SP1 binding sites, a p53 binding site and E2F binding sites were also found in this region (Fig. 1J). To exclude the effects of the other transcription factors, we created a truncated version of the full-length p21 promoter, the mini-p21 promoter, which only contained the SP1 binding sites. Lithium treatment resulted in a remarkable increase in the activity of the mini-p21 promoter. Additionally, overexpression of HDAC1 partially repressed the lithium-mediated increase in mini-p21 promoter activity (Fig. 1J). These results indicate that lithium can decrease HDAC1 levels and increase the expression of HDAC1 target genes.

Another consequence of HDAC1 down-regulation is an increase in the cellular acetylation levels. We tested the activity of the HDACs using an HDAC activity detection assay. It showed that HDACs activity gradually decreased in response to lithium (Fig. 1K).

Lithium Inhibits the Synthesis of HDAC1 Protein—It has been reported that lithium affects many targets at the transcriptional level. To determine whether the decrease in HDAC1 protein levels could be attributed to decreased HDAC1 transcription, qPCR was performed to measure the HDAC1 mRNA levels. Interestingly, the HDAC1 mRNA level was unchanged in the 293T, HeLa, and SW480 cell lines (Fig. 2, A–C). This suggests that lithium does not regulate HDAC1 at the transcriptional level.

We then assessed whether lithium affects HDAC1 protein stability. In eukaryotic cells, there are two main pathways for protein degradation: the proteasome-dependent pathway and the lysosome-dependent pathway. If lithium affects the stability of the HDAC1 protein, treatment with proteasome inhibitors or autophagy inhibitors should restore the protein levels in the presence of lithium. However, neither the 26 S proteasome inhibitor MG132 (Fig. 2D) nor the 20 S proteasome inhibitor lactacystin (Fig. 2E) prevented the decrease in HDAC1 levels induced by lithium. The autophagy inhibitors 3-methyladenine and bafilomycin also failed to inhibit the decrease in HDAC1 caused by lithium treatment (Fig. 2, F and G). Lithium also decreased HDAC1 protein levels in the presence of the pan-caspase inhibitor benzoxycarbonyl-VAD (Z-VAD; H) in the presence and absence of 30 mM LiCl.

FIGURE 2. The lithium-mediated decrease in HDAC1 occurs at the translational level. A–C, lithium did not affect HDAC1 mRNA levels. HEK 293T (A), HeLa (B), and SW480 cells (C) were treated with LiCl for the indicated times. HDAC1 mRNA was measured using qPCR, and expression was normalized to GAPDH expression. The experiments were repeated three times. The data represent the mean ± S.D. D–H, lithium did not affect HDAC1 protein stability. HeLa cells were treated with 10 μM MG132 (D), 2 mM lactacystin (Ect) (E), 5 mM 3-methyladenine (3-MA; F), 200 μM bafilomycin A1 (Baf) (G), or 10 μM benzoxycarbonyl-VAD (Z-VAD; H) in the presence and absence of 30 mM LiCl.
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FIGURE 3. A and B, cycloheximide (CHX) did not alter HDAC1 protein levels in the presence of lithium. HeLa (A) and SW480 (B) cells were treated with 0.1 mM cycloheximide in the presence or absence of 30 mM LiCl for the indicated times. HDAC1 levels were detected by Western blot analysis. C, sucrose density gradient centrifugation. HeLa cell lysates treated with 30 mM LiCl were fractionated by 10–50% (w/v) sucrose gradient centrifugation. The distributions of HDAC1 and HDAC2 mRNAs across the gradient were detected using semi-quantitative PCR.

A cell line in which the Wnt signaling pathway was blocked in the presence of lithium in both the HeLa and SW480 cells (Fig. 3, A and B). This result indicates that lithium probably regulates the translation of HDAC1. These results were also consistent with data indicating that lithium does not affect the stability of the HDAC1 protein, as shown in Fig. 2. D–H. To further confirm that lithium inhibits the translation of HDAC1, we used a sucrose density gradient centrifugation assay to examine the binding activity of HDAC1 mRNA to polysomes. HDAC1 mRNA shifted to the light polysomes fraction after lithium treatment. We did not observe changes in the HDAC2 mRNA distribution along the gradient (Fig. 3C). This result is consistent with the finding that lithium does not affect the HDAC2 protein level. Thus, we conclude that lithium inhibits the translation of HDAC1 mRNA by decreasing the binding activity of HDAC1 mRNA to polysomes.

Lithium-facilitated Down-regulation of HDAC1 Is Independent of GSK3β—Previous studies have established that GSK3β is a target of lithium (16), and GSK3β has also been identified as an upstream factor in the Wnt signaling pathway. We first tested whether the lithium-induced down-regulation of HDAC1 was mediated by GSK3β or the Wnt signaling pathway using the GSK3β-specific inhibitor SB216763. Although SB216763 markedly increased the β-catenin levels and had a greater stabilizing effect on β-catenin compared with lithium (Fig. 4A), the HDAC1 levels were not affected even when the treatment time was extended to 48 h (Fig. 4B). Similar results were also observed in the SW480 cell line in which the Wnt signaling pathway is constitutively activated; β-catenin cannot be degraded in this cell line due to a mutation in adenomatosis polyposis coli, which is important for the degradation of β-catenin (Fig. 4C). Another GSK3β inhibitor, CHIR-99021, also had no effect on HDAC1 protein levels (Fig. 4D). These results suggest that the lithium-induced down-regulation of HDAC1 is independent of the GSK3β/Wnt/β-catenin pathway.

Lithium-mediated HDAC1 Down-regulation Is Independent of CUGBP1—Next, we sought to determine whether lithium affects the factors that influence HDAC1 protein levels. It has been reported that CUG triplet repeat binding protein (CUGBP1) can enhanced HDAC1 translation (47, 48). To explore the possible involvement of CUGBP1 in the regulation of HDAC1 translation, we overexpressed CUGBP1 in 293T cells. Overexpression of CUGBP1 rescued the lithium-mediated down-regulation of HDAC1 (Fig. 5A). However, overexpression of the translation initiation factor eIF2A, which was reported to interact with CUGBP1, and its phosphorylation site mutant eIF2A (S52A) had no effect (Fig. 5B). Based on these
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A. overexpression of CUGBP1 can rescue the lithium-mediated down-regulation of HDAC1. HeLa cells were treated with 30 mM LiCl at 18 h post-transfection with CUGBP1. Exogenous CUGBP1 was examined by Western blot analysis after treatment with LCl for 24 h. B, E1F2A cannot rescue the lithium-mediated down-regulation of HDAC1. C, HEK293T cells were transfected with E1F2A or E1F2A (S52A), and 20 mM LiCl was added to the transfected HEK293T cells at 18 h post-transfection. D, lithium did not affect CUGBP1 protein levels. E, HeLa cells treated with 30 mM LiCl were analyzed by immunofluorescence with an anti-CUGBP1 antibody. The cells were also treated with the FLAG antibody. F, lithium did not affect the localization of CUGBP1. G, HeLa cells treated with 30 mM LiCl were analyzed by immunofluorescence with an anti-CUGBP1 antibody. The cells were also treated with 20 ng/ml leptomycin B (LMB) in the presence of lithium. H, lithium did not affect CUGBP1 protein levels, and CUGBP1 levels were measured by Western blot analysis. I, lithium did not affect the localization of CUGBP1. J, exogenous HDAC1 expressed from the cDNA, but not from the CDS, was decreased after lithium treatment. HeLa cells stably expressing HDAC1 cDNA or CDS were treated with 30 mM LiCl for the indicated times. Exogenous HDAC1 levels were detected by Western blot analysis using the FLAG antibody. Bottom, diagram of the different HDAC overexpression plasmids.

Recent studies reported that CUGBP1 can shuttle from the nucleus to the cytoplasm and form stress particles, and its subcellular localization is dependent on its phosphorylation status, which can change upon exposure to a variety of environmental stresses (49, 50). Therefore, we next tested whether lithium affects the subcellular localization of CUGBP1. Confocal images showed that CUGBP1 did not form granules in the cytoplasm in most of the cells treated with lithium, and the export of CUGBP1 was elevated, not inhibited by leptomycin B (LMB) (Fig. 5E). These results suggest that lithium did not affect CUGBP1 protein levels or subcellular localization. Therefore, we conclude that lithium does not down-regulate HDAC1 protein levels through CUGBP1.

Lithium Targets the Entire HDAC1 mRNA—Recent studies have shown that lithium can influence a subset of microRNA molecules (51–53). MicroRNAs regulate mRNA stability and protein translation by binding to the UTR of the mRNAs. To
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FIGURE 6. Down-regulation of HDAC1 is essential for the degradation of mutant huntingtin through lithium-induced autophagy. A and B, LiCl decreased the level of Htt590-100Q but not Htt590-100Q (K444R). Stable HeLa cell lines were treated with 15 mM LiCl for the indicated times. Ectopically expressed Htt590-100Q and Htt590-100Q (K444R) were detected using the His-tag antibody. C, p62 preferentially interacted with Htt590-100Q. Stable HeLa cell lines were treated with 15 mM LiCl for 48 h followed by immunoprecipitation (IP) with the p62 or His-tag antibodies. IB, Immunoblot. D, lithium induced the colocalization of Htt590-100Q with LC3 but not the colocalization of Htt590-100Q (K444R) with LC3. After treatment with 15 mM LiCl for 48 h, stable HeLa cell lines were analyzed by immunofluorescence with the His and LC3 antibodies using a confocal microscope. VPA was used as a positive control for colocalization. Scale bars, 20 μm. E, lithium enhanced the interaction between Htt590-100Q and p62. HeLa cells stably expressing Htt590-100Q were treated with 15 mM LiCl for 48 h followed by immunoprecipitation with the His-tag antibody and Western blot analysis for His tag and p62. F, overexpression of HDAC1 blocked the lithium-induced degradation of Htt590-100Q. SW480 cells stably expressing Htt590-100Q-His and estrogen receptor-HDAC1-FLAG were treated with or without 15 mM LiCl and 10 μg/ml tamoxifen for 60 h. Htt590-100Q and estrogen receptor-HDAC1 were detected by Western blot analysis with the His and FLAG antibodies.

examine whether microRNAs are involved in the lithium-mediated HDAC1 down-regulation, we performed qPCR analysis to detect miR-449a, a well known microRNA that targets HDAC1. If lithium decreases HDAC1 protein through a microRNA-mediated pathway, lithium treatment should increase the microRNA levels. However, our data showed that lithium significantly decreased miR-449a levels in 293T and HeLa cells; this decrease was less significant in SW480 cells (Fig. 5, F–H), suggesting that the lithium-mediated down-regulation of HDAC1 did not occur through miR-449a. To determine whether lithium affects other microRNAs that target the 3’-UTR of the HDAC1 mRNA, we cloned the HDAC1 promoter (−1170 to +397 bp) (54) and the 3’-UTR of the HDAC1 mRNA and inserted the cloned fragments into the 5′ terminus and 3′ terminus of the firefly luciferase gene; thus we constructed a reporter driven by the HDAC1 promoter and containing the 3’-UTR of the HDAC1 mRNA (Fig. 5H, bottom). Luciferase reporter assays showed that lithium treatment did not alter the luciferase activity of the reporter regardless of whether the 3’-UTR was present (Fig. 5I). Briefly, these data suggest that the lithium-facilitated down-regulation of HDAC1 is independent of the microRNAs that target the UTR of the HDAC1 mRNA.

However, the question of how lithium regulates HDAC1 mRNA remains unanswered. We know that lithium decreases the binding activity of the HDAC1 mRNA to the polyribosomes, and lithium does not target the UTR of the HDAC1 mRNA. We then wanted to know whether lithium regulates the coding sequence (CDS) region of the HDAC1 mRNA. HeLa cell lines stably expressing either the entire HDAC1 mRNA or only the CDS were constructed. Interestingly, we found that lithium decreased the level of exogenous HDAC1 expressed from the CDS and not from only the CDS (Fig. 5J). This result suggests that lithium probably targets the entire HDAC1 mRNA.

Lithium-induced Down-regulation of HDAC1 Is Essential for the Autophagic Degradation of Mutant Huntingtin and the Decrease in Cytotoxicity—Previous work showed that lithium induces the degradation of mutant huntingtin through autophagy (8). Jeong et al. (38) reported that the acetylation of mutant huntingtin at lysine 444 enhances its clearance through the autophagic-lysosomal pathway, whereas deacetylation of Lys-444 by HDAC1 inhibits its degradation. To determine whether HDAC1 is involved in the lithium-mediated degradation of mutant huntingtin, stable HeLa cell lines were constructed that express mutant huntingtin containing an N-terminal fragment of 590 amino acids and 100 glutamine repeats with an intact Lys-444 (Htt590-100Q) or the point mutation K444R (Htt590-100Q (K444R)). Although HDAC1 levels were decreased in response to lithium in both cell lines, lithium induced significant degradation of the Htt590-100Q mutant
but not the Htt590-100Q (K444R) mutant (Fig. 6, A and B). Next, we used microtubule-associated protein 1 light chain 3 (LC3) to monitor the autophagic activity in HeLa cells; LC3 is incorporated into the autophagosomal membrane when autophagy occurs. Treatment with lithium or VPA resulted in the formation of granules that co-localized with LC3 in the Htt590-100Q cell line but not in the Htt590-100Q (K444R) line (Fig. 6D). Additionally, we found that p62/SQSTM1, which interacts with LC3 and plays a crucial role in the lysosomal degradation pathway, tended to interact more with the Htt590-100Q mutant than the Htt590-100Q (K444R) mutant (Fig. 6C). Moreover, lithium significantly enhanced the interaction between Htt590-100Q and p62 (Fig. 6E).

Next, we sought to investigate whether overexpression of HDAC1 can block the lithium-mediated degradation of mutant huntingtin. An inducible SW480 cell line expressing estrogen receptor-HDAC1 was established by fusing HDAC1 to the hormone binding domain of the human estrogen receptor. We did not use HeLa cells in this experiment because these cells expressed high levels of estrogen. We observed that tamoxifen-induced overexpression of HDAC1 abolished the lithium-induced degradation of Htt590-100Q (Fig. 6F). These results reveal that down-regulation of HDAC1 is essential for the lithium-induced degradation of mutant huntingtin.

To explore the role of HDAC1 in the lithium-mediated neuroprotective effect in neural cells, we first tested whether lithium can down-regulate HDAC1 in neural cells. The rat neuroblastoma cell line ND7/23 and the human neuroblastoma cell line SK-N-SH were used in these experiments. A reduction in HDAC1 protein levels was observed in both cell lines after treatment with lithium (Fig. 7A). This result indicates that the down-regulation of HDAC1 by lithium is likely independent of the cell types.

We then constructed stable SK-N-SH cell lines expressing Htt590-100Q or Htt590-100Q (K444R). Overexpression of both mutant huntingtin constructs obviously induced cell death in the SK-N-SH stable cell lines (Fig. 7C). We then infected two stable cell lines with a lentivirus expressing a kinase-dead mutant with a deletion in the zinc binding domain: HDAC1 (ΔZn). We observed that the overexpression of HDAC1 (ΔZn) significantly decreased the levels of Htt590-100Q, but not Htt590-100Q (K444R), in stable cell lines after 7 days (Fig. 7B). Additionally, overexpression of HDAC1 (ΔZn) decreased cell death in the Htt590-100Q stable cell line but not in the Htt590-100Q (K444R) stable cell line (Fig. 7, C and D). To further confirm the cell toxicity induced by mutant huntingtin, we performed staining experiments to examine cleaved caspase-3 in the stable cell lines. Both mutant huntingtin pro-

FIGURE 7. The lithium-induced down-regulation of HDAC1 is essential for the degradation of mutant huntingtin and the decrease in cytotoxicity. A, the rat neuroblastoma cell line ND7/23 and the human neuroblastoma cell line SK-N-SH were treated with 20 mM LiCl for the indicated times, and endogenous HDAC1 levels were detected by Western blot analysis. B, the HDAC1 dominant negative mutant decreased the levels of Htt590-100Q but not the levels of Htt590-100Q (K444R). SK-N-SH cells stably expressing Htt590-100Q or Htt590-100Q (K444R) were infected with lentivirus expressing the dominant negative mutant HDAC1 (ΔZn), and these cells were cultured and passed for 10 days. Ectopically expressed mutant huntingtin and mutant HDAC1 levels were detected by Western blot analysis with the His-tag and FLAG-tag antibodies. C, morphological changes in the different stable cell lines used in B. EV, empty vector. D, quantification of the dead cells in C. Three fields were scored. E, lithium attenuated the cell death induced by mutant huntingtin. SK-N-SH cells stably expressing Htt590-100Q or Htt590-100Q (K444R) were treated with 5 mM LiCl or 0.5 mM VPA for 3 days, and the levels of cleaved caspase-3 were examined by immunofluorescence. Scale bars, 100 μm. F, quantification of the dead cells in E. Three fields were scored. *, p < 0.05; **, p < 0.01; ***, p < 0.001. n.s., not significant.
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proteins induced cleavage of caspase-3. However, lithium treatment decreased the levels of cleaved caspase-3 in the Htt590-100Q stable cell line but not in the Htt590-100Q (K444R) stable cell line (Fig. 7, E and F). However, VPA had weaker effects than lithium. We supposed that SK-N-SH is a neuroblastoma cell, and it is known that VPA can induce apoptosis in several tumor cells (55–57). Additionally, we observed that Htt590-100Q (K444R) was more likely to localize in the nucleus when compared with Htt590-100Q in the SK-N-SH cells; however, there was no difference in the HeLa cells. Therefore, we conclude that HDAC1 is essential for the lithium-mediated degradation of mutant huntingtin and the decrease in cytotoxicity.

DISCUSSION

Lithium was administered as an antimanic drug during the 19th century and has been used for bipolar disorder in the clinical setting since the 1950s. Lithium is the lightest metal in the periodic table, and it is also a powerful and multifunctional ion. Lithium affects gene expression, mRNA translation, and post-translational modifications of proteins by directly or indirectly modulating a large number of cellular compounds. For example, recognized targets of lithium include GSK3β, IMPase (inositol monophosphatase 1), Akt, ERK, p53, adenosine monophosphate-activated protein kinase, BDNF, and Tau. Wang et al. (14) also reported that lithium greatly enhances the generation of induced pluripotent stem cells by increasing the expression and activity of Nanog and down-regulating LSD1. The extremely large number of targets makes it difficult to clearly analyze the therapeutic mechanisms of lithium. Despite several decades of effort, the mechanisms of lithium action have still only been partially elucidated. The cellular membrane permeability and the minimal protein binding activity of lithium make it especially difficult to understand.

GSK3β plays an important role in neurodegeneration (58, 59). Lithium and the GSK3β-specific inhibitors SB-216763 and CHIR-99021 significantly inhibit GSK3β (Fig. 4) and affect Wnt target genes, such as β-catenin and DKK-1 (data not shown). However, only lithium decreases HDAC1 protein levels. These results indicate that the down-regulation of HDAC1 caused by lithium is not due to its inhibition of GSK3β. Other signaling pathways affected by lithium including the p38/MAPK, ERK (60), MNK1/2, PI3K/ AKT (61), adenosine monophosphate-activated protein kinase (62), and mTOR signaling pathways are also reported to regulate protein synthesis. We screened these pathways by Western blot analysis using several small chemical molecules to evaluate whether these signaling pathways and related components were involved in the inhibitory effect of lithium on HDAC1. However, our results showed that these small chemical molecules had no effect on the lithium-induced reduction of HDAC1 protein levels. Additionally, inhibition of PP1/PP2A, tyrosine kinase, and sphingosine 1-phosphate by okadaic acid, Genistein, and FTY720 also had no effect on HDAC1 protein levels (data not shown).

Kim et al. (63) reported that HDAC1 was exported from the nucleus in response to pathological conditions and that this process was dependent on calcium ion influx, which was inhibited by EDTA and leptomycin B. We also tested these compounds and found that EDTA and leptomycin B had no effect on HDAC1 protein levels (data not shown).

CUGBP1 is a bifunctional molecule that binds to the 5′-UTR of some CUG repeat mRNAs and increases their translation (50, 64, 65); however, this protein has also been shown to bind to GU-rich elements in the 3′-UTR of mRNAs and inhibit their translation (66, 67). Additionally, Sofola et al. found that overexpression of human CUGBP1 suppresses the neurodegenerative eye phenotype in transgenic flies (75). HDAC1 is regulated by CUGBP1 at the translational level (47, 48). However, we did not observe any changes in the protein levels or subcellular localization of CUGBP1 after treatment with lithium. This suggests that CUGBP1 is not involved in the lithium-mediated down-regulation of HDAC1. Interestingly, we found that lithium can down-regulate exogenous HDAC1 expressed from a vector containing the full-length cDNA but not from a vector containing only the coding region (Fig. 5). We concluded that lithium targets the entire mRNA of HDAC1; in other words, lithium may affect the secondary structure or the nuclear export of HDAC1 mRNA. This mechanism requires further investigation.

Previous studies have suggested that acetylation homeostasis is impaired during neurodegeneration. In most neurodegenerative diseases, the activity of histone acetyltransferases, such as cAMP-response element-binding protein (CREB)-binding protein and p300 is lost (39), which leads to a decrease in global acetylation levels in neurons. Thus, reinstating acetylation homeostasis is a reasonable means of treating neurodegenerative diseases in accordance with the observed neuroprotective role of some HDAC inhibitors (33, 68). In the present study we demonstrated that HDAC1 is important for the clearance of mutant huntingtin under the influence of lithium. We used the long mutant huntingtin Htt590-100Q construct, which was used by Jeong et al. (38) rather than a huntingtin exon 1 fragment with 74 or 100 polyglutamine repeats, which has been used in other experiments (8, 69, 70). Jeong et al. (38) reported that short mutant huntingtin and long mutant huntingtin might exist in different forms in the cell. Long mutant huntingtin does not tend to form visible aggregates; this phenomenon is more likely to occur with high molecular weight huntingtin. We showed that Htt590-100Q, but not the acetylation-resistant Htt590-100Q (K444R), could be cleared by lithium-induced autophagy. However, previous work showed that lithium induced the clearance of Htt171-74Q through autophagy (8, 71). Short mutant Htt171-74Q has no acetylation sites, which suggests that degradation of the long mutant huntingtin is more sophisticated. Additionally, we observed that Htt590-100Q (K444R) was more likely to localize in the nucleus of 5K-N-SH cells compared with Htt590-100Q (Fig. 7D). This result suggests that deacetylation might be related to the nuclear export of mutant huntingtin. Interestingly, this phenomenon was not observed in HeLa cells. We also observed that Htt590-100Q (K444R) formed more inclusion bodies than Htt590-100Q in HeLa cells (data not shown). However, these proteins did not show any difference in cytotoxicity. Further studies should be focused on how and why the acetylated mutant huntingtin is prone to autophagic degradation. We hypothesize that lithium affects the degradation of mutant huntingtin in two ways: through the down-regulation of HDAC1 and through the
induction of autophagy. Inhibition of HDACs not only induces autophagy (72–74) but also increases acetylation and enhances the clearance of mutant huntingtin. These results provide an explanation for the similar clinical efficacy of lithium and VPA and also confirm the crucial role of HDAC1 in neurodegenerative diseases. Additional studies utilizing animal models and clinical samples should be undertaken to further examine these points.

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