

Supporting Information

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SI Materials and Methods

Tissue Culture and in Vitro Histone Deacetylase Inhibitor Treatment.

Primary human fetal astrocyte tissue cultures were purchased and maintained according to the manufacturer's protocol (ScienCell). An in vitro scratch assay was used for the injury of astrocytes as previously described (1, 2). Monolayer cells were scraped with a sterile pipette tip. Cells were kept in an incubator with fresh medium for 4 h to recover. Astrocytes were then treated for 24 h with the final drug concentrations dissolved in DMSO:LB-205 2.5 μ M and 5.0 μ M, suberoylanilide hydroxamic acid 2.5 μ M, and valproic acid 4.0 mM. Cellular morphology and wound healing were measured and recorded. Cell pellets were collected for protein or DNA extraction and cells on chamber slides were fixed for immunofluorescence assay.

Cell Cycle Analysis Flow Cytometry and Apoptosis Assay. Cultured astrocytes were fixed with 70% (wt/vol) ethanol overnight at 20 °C. Fixed cells were stained with 10 mg/mL propidium iodide and 1 mg/mL RNase for 30 min, and cell cycle was analyzed by FACS. Additionally, cultured astrocytes were labeled with Hoechst 33342 and propidium iodide (Vybrant Apoptosis Assay Kit no. 5; Molecular Probes) according to the manufacturer's guidelines. Cells were analyzed using a dual-laser FACS Vantage SE flow cytometer (Becton Dickinson). Hoechst 33342 was excited by using a 351-nm UV laser, and its emission was captured with a band-pass filter (450–720 nm). Cell Quest Acquisition and Analysis software (Becton Dickinson) was used to acquire and quantify fluorescence signal intensities and to graph data as bivariate dot-density plots.

Animals and in Vivo Histone Deacetylase Inhibitor Treatment. Surgery. Animal experiments were approved for use and care of animals under the protocol guidelines of the National Institutes of Health Animal Care and Use Committee. Sprague–Dawley rats (aged 6–8 wk, male) were purchased from Harlan Laboratories. Animals were randomized into three groups: sham ($n = 6$), traumatic brain injury (TBI; $n = 6$) and TBI treated with LB-205 ($n = 8$). Animals were subjected to controlled cortical impact TBI as previously described (3). Briefly, animals were anesthetized with isoflurane (1.5%) in a 1:1 oxygen–nitrous oxide gas mixture and placed in a stereotaxic device. A 5 \times 5-mm craniotomy was placed in the right parietal bone with a handheld trephine. The impactor tip was regulated down to the dural surface, and a penetrating contusion was made over the right frontal cortex at 2.1- to 2.2-mm depth at 5 m/s velocity. The burr hole was covered with a bone flap and sealed with Jet Denture Repair. Following standard postsurgical analgesia, each animal was given buprenorphine at 0.01 to 0.05 mg/kg (i.p. injection). All rats were allowed to recuperate after surgery before return to cages. Animals were observed postsurgery for appropriate incision healing and monitored daily for clinical and behavioral signs.

Drug administration. LB-205 was dissolved in DMSO at a concentration of 20 mg/mL. Final DMSO concentration was 50% in sterile saline solution for a 10 mg/kg i.p. injection. An equal volume of 50% DMSO in sterile saline solution was injected into rats in sham and TBI groups. Rats were given LB-205 (10 mg/kg) or vehicle injections at 4 h, 24 h, and 48 h after TBI or sham surgery.

Motor testing. Cross-beam balance examination is a test of motor coordination and cerebral function. Animals were allowed to walk along the cross-beam bar (1 m length) to test balance and motor skill. The test was performed before surgery (day 0) and after

surgery on days 1, 3, and 7. A skill score was recorded as percentage of cross-beam successfully navigated: animal can maintain balance and cross the 1 m bar (100%); animal can traverse 10 cm to 90 cm (10–90%); or animal can traverse less than 10 cm (0–10%).

Tissue preparation. Animals were killed at 1 or 2 wk. Rat brains were collected for examination of gross appearance. Brain, heart, lung, liver, spleen, kidney, and pancreas tissues were collected. Lesioned areas were surgically extracted. Normal brain and lesioned tissue were stored at -80 °C in optimal cutting temperature compound (Sakura Finetek) for protein biomarker analysis. Paraffin slides were obtained for histological analysis (American Histolabs).

Immunofluorescence. Slides were fixed with HistoChoice (Mandel) and labeled overnight with primary antibodies. Nuclei of cells were counterstained with DAPI (Sigma-Aldrich). Staining was visualized with an LSM 510 confocal microscope (Carl Zeiss). Primary antibodies were as follows: GFAP, and Ki-67 (1:200; Abcam); and nestin (1:200; Santa Cruz Biotechnology).

Protein Extraction and Western Blotting. Cell pellets were lysed in RIPA buffer (Thermo Fischer Scientific) with Halt Protease Inhibitor (Thermo Fischer Scientific), sonicated, and centrifuged. Protein quantity was determined in the supernatant by Bio-Rad Protein Assay. Equal amount of proteins were denatured at 95 °C for 5 min in protein loading buffer, and loaded on a Nu-PAGE 4% to 12% Bis-Tris gel (Invitrogen) and transferred to PVDF membranes (Invitrogen). Membranes were blocked in 5% dried skim milk in PBST and blotted with primary antibody. Primary antibodies were as follows: phospho-neurotrophic tyrosine kinase receptor type 1 (p-TrkA), p75 neurotrophin receptor (NTR), phospho-protein kinase B (p-AKT) (473), phospho-JNK, caspase-3, poly ADP ribose polymerase (PARP), NF- κ B (p65), and H2A.X (1:1,000; Cell Signaling); nerve growth factor (NGF), nestin, β -actin (1:1,000; Santa Cruz Biotechnology); acetyl-histone 1.4, acetyl-histone 3, and acetyl-histone 4 (1:1,000; Millipore); and nuclear receptor co-repressor-1 (NCoR) (1:1,000; Abcam).

Whole-Genome Expression Microarray Analysis. Gene expression microarray was performed by using Human LncRNA Microarray V2.0 (ArrayStar) as previously described (4). Sample labeling and array hybridization were performed according to the Whole Human Genome Oligo Microarray (4 \times 44K, Agilent Technologies). Feature Extraction Software (Agilent) gene expression data were imported into GeneSpring GX (software v12.1) for normalization. Two-sample t tests assuming equal variance ($P \leq 0.05$) with a fold-change screening of at least twofold were used to identify differentially expressed genes.

Quantitative Real-Time PCR. Total RNA was extracted using RNeasy mini kit (Qiagen) and cDNA was synthesized using SuperScript III First-Strand Synthesis SuperMix (Invitrogen). RT-PCR was performed by using an Eco RT PCR System (Illumina) and SYBR Green Master Mix (Applied Biosystems).

Statistical Analysis. Two-way repeated-measures ANOVA and ordinary one-way ANOVA tests were used to determine statistical differences. A Holm–Sidak method for multiple comparisons post hoc test was used to determine data points with significant differences. Data were considered significant at $P \leq 0.05$.

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