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Transcriptional activation of NAD$^+$-dependent protein deacetylase SIRT1 by nuclear receptor TLX

Naotoshi Iwahara [a], Shin Hisahara [a,b], Takashi Hayashi [a,b], Yoshiyuki Horio [a,*]

[a] Department of Pharmacology, Sapporo Medical University, Sapporo 060-8556, Japan
[b] Department of Neurology, Sapporo Medical University, Sapporo 060-8556, Japan

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ABSTRACT

An orphan nuclear receptor TLX is a transcriptional repressor that promotes the proliferation and self-renewal of neural precursor cells (NPCs). SIRT1, an NAD$^+$-dependent protein deacetylase, is highly expressed in the NPCs and participates in neurogenesis. Here, we found that TLX colocalized with SIRT1 and knockdown of *TLX* by small interfering RNAs decreased SIRT1 levels in NPCs. TLX increased the SIRT1 expression by binding to the newly identified TLX-activating element in the *SIRT1* gene promoter in HEK293 cells. Thus, TLX is an inducer of SIRT1 and may contribute to neurogenesis both as a transactivator and as a repressor.

TLX, a homolog of *Drosophila tailless*, is expressed in neural precursor cells (NPCs) and retinal precursor cells, and helps to maintain them in an undifferentiated, proliferative state [1,2]. *TLX*$^{-/-}$ mice show severe limbic defects, progressively increasing violent behavior and a marked decrement in spatial learning [3]. TLX is also expressed in retinal proangiogenic astrocytes, where it contributes to the formation of the fibronectin scaffold for angiogenesis [4]. In *Drosophila*, *tailless* participates in pattern formation [5,6], although the contribution of mammalian TLX to segmentation is not clear. TLX represses gene expression by binding a consensus site, AAGTCA (the TLX-binding site), in the promoter region [7]. An exception to TLX's target is the *retinoic acid receptor β2* (*RARβ2*) gene: TLX potentiates the retinoic acid-dependent transactivation of *RARβ2* [8].

An NAD$^+$-dependent histone/protein deacetylase SIRT1 regulates glucose and fat metabolism and may contribute to longevity in calorie restriction [9]. *SIRT1*$^{-/-}$ mice infrequently survive postnatally and exhibit abnormalities including exencephaly and retinal defects [10]. SIRT1 is highly expressed in NPCs and contributes to neurogenesis [11].

Here, we show that the *SIRT1* gene is transactivated by TLX via a TLX-activating element.

Materials and methods

Antibodies. The antibodies used were anti-SIRT1 (1:500; [12] or sc-15404 Santa Cruz Biotechnology), anti-TLX (1:200 or 1 μg/sample; H6506 or H6510 Perseus Proteomics, Inc.), anti-GAPDH (1:10,000; Sigma–Aldrich), anti-5-bromo-2′-deoxyuridine (BrdU) (1:500; Sigma–Aldrich), and anti-RNA polymerase II (1 μg/sample; Millipore).

Cell culture and transfection. The Animal Welfare Guidelines of Sapporo Medical University were followed in all the experiments. NPCs were isolated from embryonic day (E) 14.5 ddY mice (Sankyo Labo Service) as described previously [11]. *TLX*-siRNAs of a duplex of 5′-cucucaacagcuacauucatt-3′ and 5′-ugaauguagcuguugagagtt-3′ (*TLX*-siRNA1) or a duplex of 5′-gcuguaucuggcaugaauatt-3′ and 5′-uauucaugccagauacagctt-3′ (*TLX*-siRNA2), or negative *control*-siRNAs (B-Bridge International) (100 nM each) were electroporated using mouse NSC nucleofector solution (Amaxa GmBH, Germany) once a day for 2 days. HEK293 cells and PC12 cells were transfected with Lipofectamine LTX (Invitrogen). BrdU (20 μM) was added to the culture medium 12 h before harvest. Human TLX cDNA clone

Abbreviations: NPCs, neural precursor cells; siRNA, small interfering RNA; BrdU, 5-bromo-2′-deoxyuridine; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; RA, retinoic acid.

* Corresponding author. Address: Department of Pharmacology, Sapporo Medical University, South 1, West 17, Chu-ouku, Sapporo 060-8556, Japan. Fax: +81 11 612 5861.
E-mail address: horio@sapmed.ac.jp (Y. Horio).

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was purchased from Open Biosystems. RT-PCR was carried out as described previously [11]. The primers were: 5′-gaacctttgcctcatcta ca-3′ and 5′-agccgcttactaatctgctc-3′ for *SIRT1*; 5′-tacttccgtggacac aaga-3′ and 5′-tcttccaaaagcatcagctg-3′ for *TLX*; 5′-accacagtccatg ccatcac-3′ and 5′-tccaccacctgttgctgta-3′ for *GAPDH*.

Blot analyses and immunostaining. A Mouse Brain Aging Blot (Seegene) was probed with ^{32}P-labeled mouse *SIRT1* cDNA as described previously [12]. Western blot analysis and immunostaining were performed as described previously [13]. NIH Image was used to analyze the data.

Luciferase assay. The promoter region of the mouse *SIRT1* gene (1.9 kb) was amplified by PCR using the primer set of 5′-ctgtcccat catgccaggctcctg-3′ and 5′-cttccaactgcctctctggccctc-3′, from a *SIRT1* phage clone isolated from a mouse genomic DNA library (BD Biosciences). Deletion constructs were generated using PCR or synthetic oligonucleotides and inserted into KpnI–SacI-digested *pGL3* vector (Promega). The nucleotide sequence was confirmed by sequencing. Plasmid DNA (0.4 μg/well) was transfected with *PRL-TK* (20 ng/well) and *PRL-CMV* (1 ng/well) (Promega). The luciferase activity was assessed using the Dual-Luciferase Reporter Assay System (Promega). The data were collected from at least three independent experiments.

Chromatin immunoprecipitation (ChIP) assay. The EZ ChIP Chromatin Immunoprecipitation kit (Millipore) was used as described previously [11]. Primers used were 5′-cccgccacgtgacccgta gtgttgtggtc-3′ and 5′-tctctccgcggcctcttgcggagcggctc-3′ for the human *SIRT1* promoter region. Another primer set, 5′-cccgccacgtgacc cgtagtgttgtggtc-3′ and 5′-gaggggggagccgccgggctgaagggcgag-3′, was also used.

Statistics. Data are presented as the means ± SEM. Differences were compared using Student's *t*-test. Multiple group comparisons were performed with one-way ANOVA. *p* values less than 0.05 were considered significant.

Results

TLX mRNA is first expressed in the mouse brain at E8, peaks at E13.5, and decreases again by E16; however, its expression again increases after birth, and it is high in the adult brain [14]. Immunostaining shows that SIRT1 expression is high around E12.5, and that it decreases by E16.5 [12]. Northern blot analysis showed that brain *SIRT1* mRNA expression was low at peri-parturition, gradually increased, and was high in the adult (Fig. 1A). Thus, the changes in the *SIRT1* mRNA levels during development are very similar to those

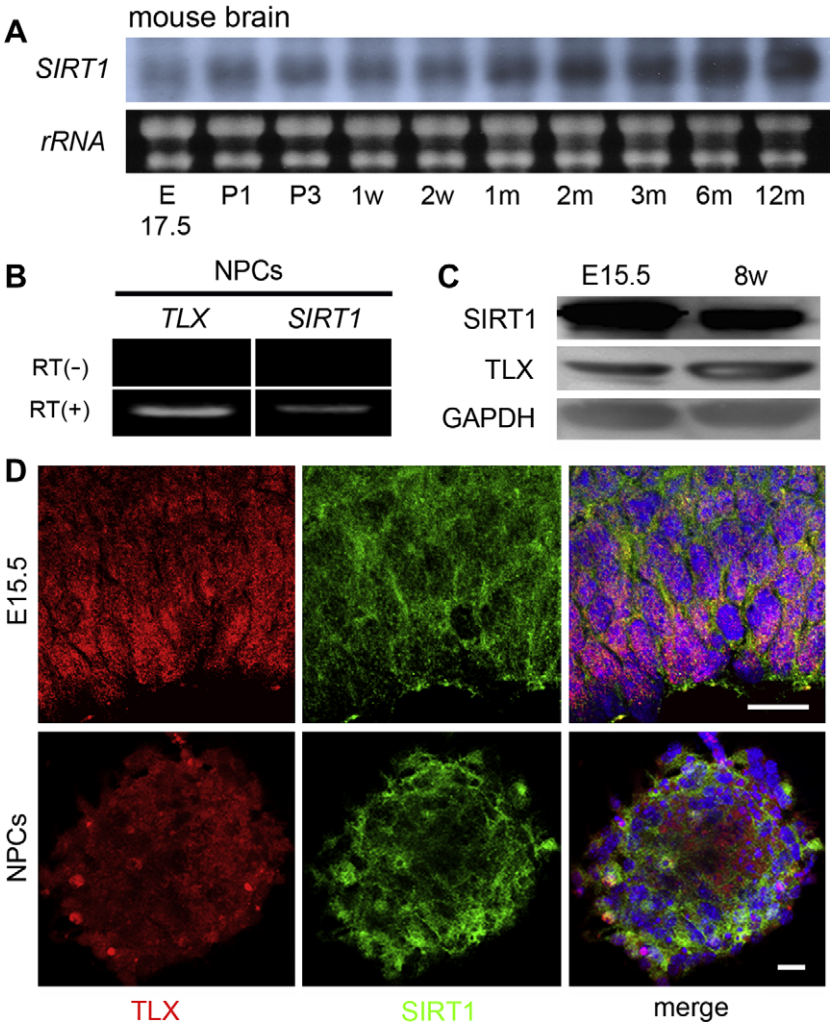

Fig. 1. Colocalization of SIRT1 and TLX in the brain. (A) Northern blot analysis of *SIRT1* mRNA in the development of mouse brain. rRNA, ribosomal RNAs. (B) RT-PCR of *TLX* and *SIRT1* mRNAs in cultured NPCs. RT, reverse transcription. (C) Western blot analysis of E15.5 and adult (8w) mouse brain. (D) Immunostaining of TLX (red) and SIRT1 (green) in the SVZ of the E15.5 mouse brain (E15.5) and a cultured neurosphere (NPCs). Nuclei were stained with Hoechst 33342 (blue). E, embryonic day; P, postnatal day; w, week(s); m, month(s). Scale bars: 20 μm. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)