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TRANSCRIPTION LEVELS OF SIRTUIN FAMILY IN NEURAL STEM CELLS AND BRAIN TISSUES OF ADULT MICE

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Abstract

Neural stem cells (NSCs) has been used as a well-known model to investigate apoptosis, differentiation, maintenance of stem cells status, and therapy of neurological disease. The C17.2 NSCs line was produced after v-myc transformation of neural progenitor from mouse cerebellar cortex. Sirtuin family plays important roles involved in neuronal differentiation, genomic stability, lifespan, cell survival. However, little is known about gene expression variation of sirtuin family in C17.2 NSCs, primary NSCs, and different brain tissues in adult mice. Here, we confirmed that the mRNA expression levels of sirt2, 3, 4, 5, and 7 in E14.5 NSCs were significantly higher than in C17.2 NSCs, whereas that sirt 6 displayed an opposing mode. Moreover, a higher mRNA level of sirtuin family was observed in the adult mouse brain compared to C17.2 NSCs. In addition, histone deacetylase (HDAC) inhibitors nicotinamide and Trichostatin A (TSA) were used to explore differential changes at the transcriptional level of sirtuins. Results indicated that the expression of sirt1, sirt5 and sirt6 was significant downregulated by nicotinamide treatment. Whereas, a significant downregulation in sirt1 and sirt3 and a significant upregulation in sirt2, sirt4, sirt6, and sirt7 were observed in the treatment of TSA. Thus our studies indicate different sirtuin mRNA expression profiles between C17.2 NSCs, E14.5 NSCs and brain tissues, suggesting the transcriptional regulation of sirtuin family could be mediated by different histone acetylation.

Key words: Sirtuin, Neural stem cells, Brain tissues, Histone acetylation.

Article information

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INTRODUCTION

Neural stem cells (NSCs) have be defined by their ability to self-renew and the biological potential to differentiate into the three major cell types, neurons, oligodendrocytes, and astrocytes, and thus play a key role in the development and maturation of the nervous system. NSCs have promise for treatment of neurological diseases through transplantation therapy or recruitment of endogenous precursors (38). The developmental potential of C17.2 NSCs line is resemble to endogenous neural progenitor/stem cells in that they are self-renemal and capable of differential into all neural cell types (29). C17.2 NSCs line has been demonstrated as a well-known model to investigated apoptosis, signal transduction, differentiation, maintenance of stem cells status, drug testing, and therapy of neurological disease (23,24,36). Some reports illustrated that energy metabolism is a key modulator of neural stem cell, such as stem cell self-renewal, multipotency, differentiation and aging (1,28).

The proteins of sirtuin (sirt1-7) family are a class of nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylase that play a key role in central physiological regulation such as gene transcription regulation, metabolic regulation, development, oncogenic, circadian rhythm, and cellular senescence (4,9,11,20,27). The sirt1-7 were categorized as class III HDAC due to their histone deacetylated activity that were homologous to the yeast protein silent information regulator 2 (sir2), a NAD+-dependent HDAC (14). Their mRNA transcription analyses elucidated that they were associated with numerous expression changes including transcription factors and metabolism and energy-related genes from neural stem/progenitor cells to a differentiated neural-related cells (8,16). Sirtuin affect gene expression through regulation of their substrates and activities. For example, sirt1 can deacetylate histone H1 at lysine 26 (H1K26), H3K9, and H3K56; sirt6 can deacetylate H3K9 and H3K56 (6,18,28,33). The members of sirtuin family vary widely in sub-cellular localization. Sirt1, sirt6, and sirt7 were mainly localized to nucleus; sirt2 was concentrated in the cytoplasmic; sirt3, sirt4, and sirt5 were predominantly present in mitochondria (22).

In this study, we explored the different expression levels of sirt1-sirt7 in C17.2 NSCs and E14.5 NSCs as well as adult mouse brain. Our results confirmed different sirtuin mRNA expression profiles and regulation by HDAC inhibitors. It contributes new insights into the sirtuin family function.

MATERIALS AND METHODS

Animals and tissues

C57BL/6J mice were mated, the day that vaginal plug was observed as embryonic day 0 (E0). Primary NSCs were got from mouse fetal brains on embryonic day 14.5 (E14.5). Four different brain areas contained cerebral cortex, hippocampus, cerebellar cortex and olfactory bulb were removed from mature male mice brain of the inbred line C57BL/6J aged 2.5-3 months. The care and treatment of animals used in the present study in accordance with the guidelines for the use of laboratory animals of European Community.

Neural stem cell culture

Mouse NSCs line C17.2 was cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM, Gibco)

with 10% fetal bovine serum (FCS, Gibco), 5% horse serum (HS, Gibco), in a humidified 5% CO_2 and 95% air at 37°C (29). Primary NSCs were isolated from brain of E14.5 C57BL/6J mices according to methods described previously (35). The primary neurosphere (E14.5 NSCs) were cultured in Dulbecco's modified Eagle's medium/F12 medium (Gibco) with N2 and B27 supplements that contained penicillin (100U/ml), streptomycin (100ug/ml), basic fibroblast growth factor (20ug/ml), and epidermal growth factor (20ng/ml) (36).

C17.2 exposure to nicotinamide and TSA

The drug concentrations of nicotinamide and TSA were managed according to early our study (37). HDAC inhibitors nicotinamide and TSA were studied to analyze their effects on the transcription profile of SIRT1-7 mRNA. C17.2 NSCs were precultured for 24 hours and then cultures with nicotinamide (20mM) and TSA (250nM) for 24h. C17.2 NSCs were administered through direct dilution nicotinamide or TSA into the culture medium, and an equivalent volume of vehicle was added to control cultures.

RNA extraction and cDNA synthesis

Total RNA was extracted from four brain different tissues (cerebral cortex, hippocampus, cerebellar cortex and olfactory bulb), E14.5 NSCs and C17.2 NSCs using the RNAiso™ Plus kit according to the supplier's specifications. Spectrophotometer (Eppendorf, Hamburg, Germany) was used to determined the concentration of RNA at 260/280 nm. cDNA was synthesized using 5ug of total RNA from different tissues with DNase I 37°C for 30 min, ReverTra Ace and oligo(dT) were used for first-stand synthesis following manufacturer's instructions and was stored at -80°C until used.

Quantitative RT-PCR and RT-PCR

Real-time RT-PCR analysis was performed in BioRad mini with SYBR Green Real-time PCR Mix according to the manufacturer's instructions except Sirt4. Semi-quantitative RT-PCR was used to sirt4 mRNA expression analysis. The primer sequences and annealing temperatures were used as follows: sirt1 sense, GCAACAGCATCTTGCCTGAT; sirt1 antisense, GTGCTACTGGTCTCACTT(62°C); sirt2 sense, CTTCCTGGGCATGATGAT; sirt2 antisense, ACCCTGACTGGGCATCTAT(62°C); sirt3 sense, CAGCAACCTTCAGCAGTA; sirt3 antisense, CCGTGCATGTAGCTGTTA (62°C) (13); sirt4 sense, TGACGGAGCTCCACGGATGC; sirt4 antisense,ACACGCCGGTGCACAAAGTCA (65°C); sirt5 sense, GTCATCACCCAGAACATTGA; sirt5 antisense, ACGTGAGGTCGCAGCAAGCC (62°C) (26); sirt6 sense, TCCCAAGTGTAAGACGCAGT; sirt6 antisense, GTTGCAGGTTGACAATGACC (65°C); sirt7 sense, CCCCGGACCGCCATCTCA; sirt7 antisense,ATCTCCAGGCCCAGTTCATTCAT (65°C) (34); β-actin sense, TTGCAGCTCCTTCGTTGC; β-actin antisense, CACGATGGAGGGGAATACAG (60°C) (26); GAPDH sense,GGTGAAGGTCGGTGTGAACG; GAPDH antisense, CTCGCTCCTGGAAGATGGTG (57°C); β-actin and GAPDH as internal reference in order to normalize the mRNA expression levels. The generation of specific PCR products was confirmed by melting curve analysis. $2^{-\Delta\Delta CT}$ method was used to quantify the relative mRNA express levels. To further analyze transcription level of sirtin gene family the cDNA was amplified in a total volume of 20 ul with 28 cycles of PCR. All PCR products were confirmed on 1.5% agarose gels with ethidium bromide.

Statistical analysis

All data were expressed as the means ± S.E.M. (standard error of the mean). Student's t-test was used to determine the significance of differences between two groups, while one-way analysis of variance (ANOVA) was used when there are more than two groups. P-value <0.05 was considered to be statistical significance.

RESULTS

Transcription levels of Sirtuin family in neural stem cells and brain tissues of adult mice

To evaluate the transcription patterns of sirtuin family members, C17.2 NSCs and E14.5 NSCs were cultured in standard conditions (Fig. 1). Results showed that mRNA expression level of sirt2, sirt3, sirt4, sirt5 and sirt7 was significantly increased in E14.5 NSCs. In contrast, sirt6 was exhibited a significant decrease in E14.5 NSCs. No remarkable difference in sirt1 expression was found between E14.5 NSCs and C17.2 NSCs (Fig. 2). As shown in Fig. 3, we observed an overall down-regulation of sirtuin genes in C17.2 NSCs compared with four brain tissues (cerebral cortex, hippocampus, cerebellar cortex and olfactory bulb) and E14.5 NSCs except sirt6. Especially, we observed a increase on the transcription levels of sirt1, sirt2, sirt4, and sirt5 in cerebral cortex and olfactory bulb than cerebral cortex and hippocampus.

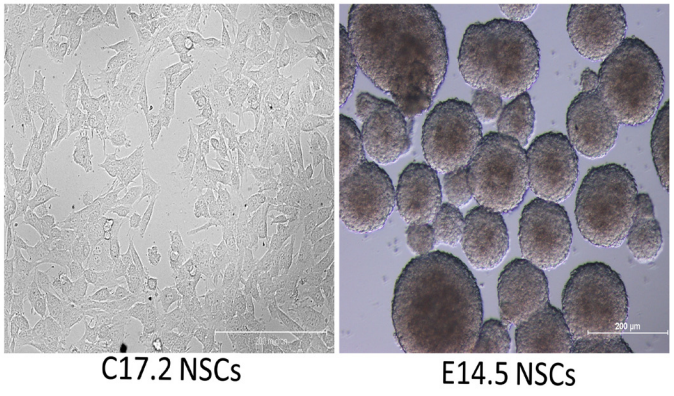

Figure 1. C17.2 NSCs and E14.5 NSCs were cultured in standard media, respectively.

Nicotinamide and TSA modulated transcription of Sirtuin family

Nicotinamide, an inhibitor, is used to inhibit class III histone deacetylases and is involved apoptosis, aging, cell division regulation, genomic silencing, DNA damage repair (10).TSA is also an inhibitor for both type I and type II histone deacetylases that involved in regulation transcriptions of gene in cell development and differentiation (5). To gain further insights into the gene expression regulation of sirt1-sirt7, we investigated the effects upon mRNA transcription levels of sirtuin family by nicotinamide and TSA in the C17.2 cell line. Our results demonstrated that when exposed to nicotinamide (20mM), the expression of sirt1, sirt5 and sirt6 showed a statistical significant down-regulation. Although sirt3 expression was down-regulated

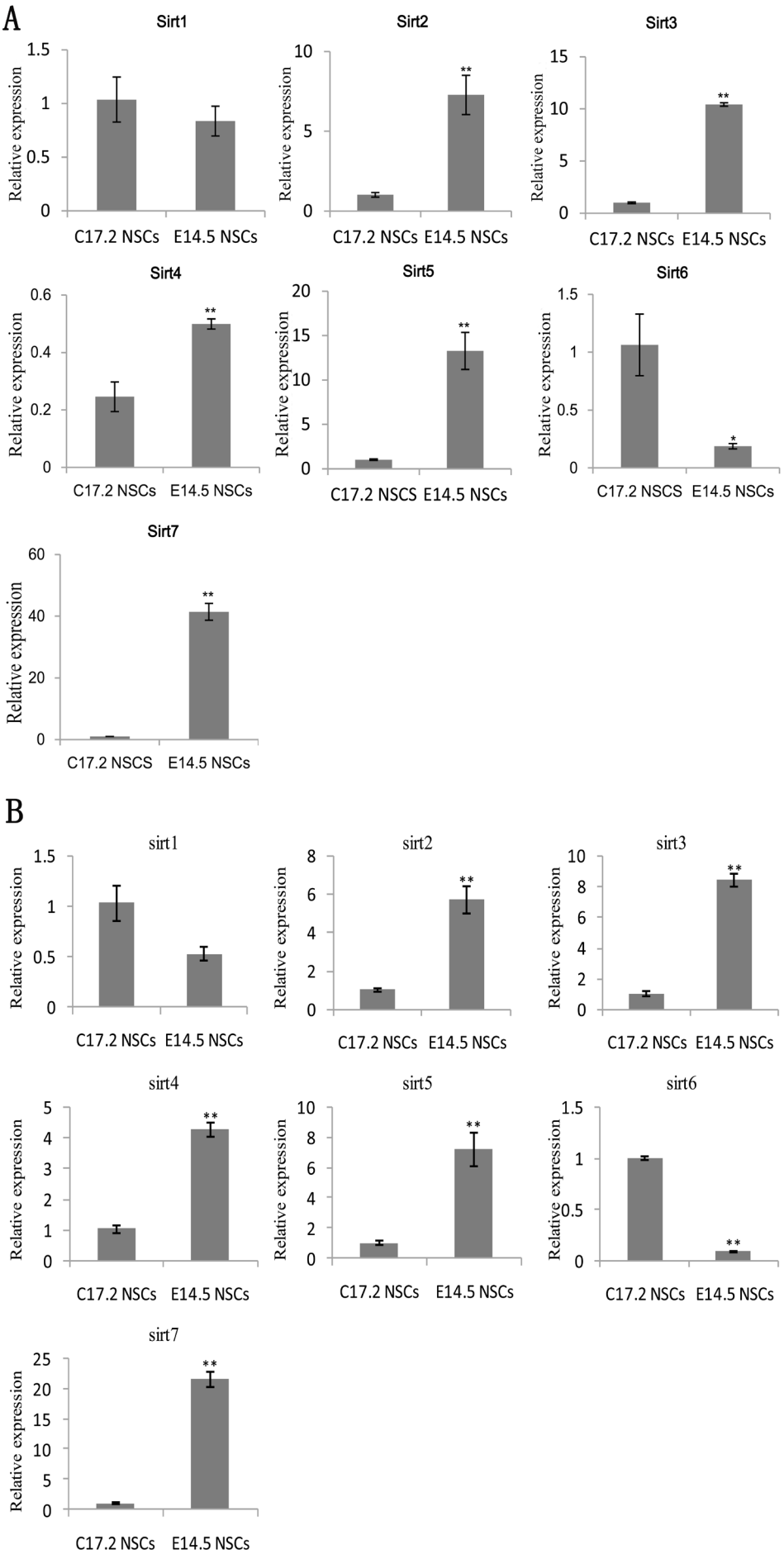

Figure 2. Sirtuin family was subjected to quantitative PCR analysis from C17.2 NSCs and E14.5 NSCs. Quantitative data (means±SEM) which are based on three independent experiments were normalized to β-actin (A) and GAPDH (B). *p<0.05;**p<0.01.

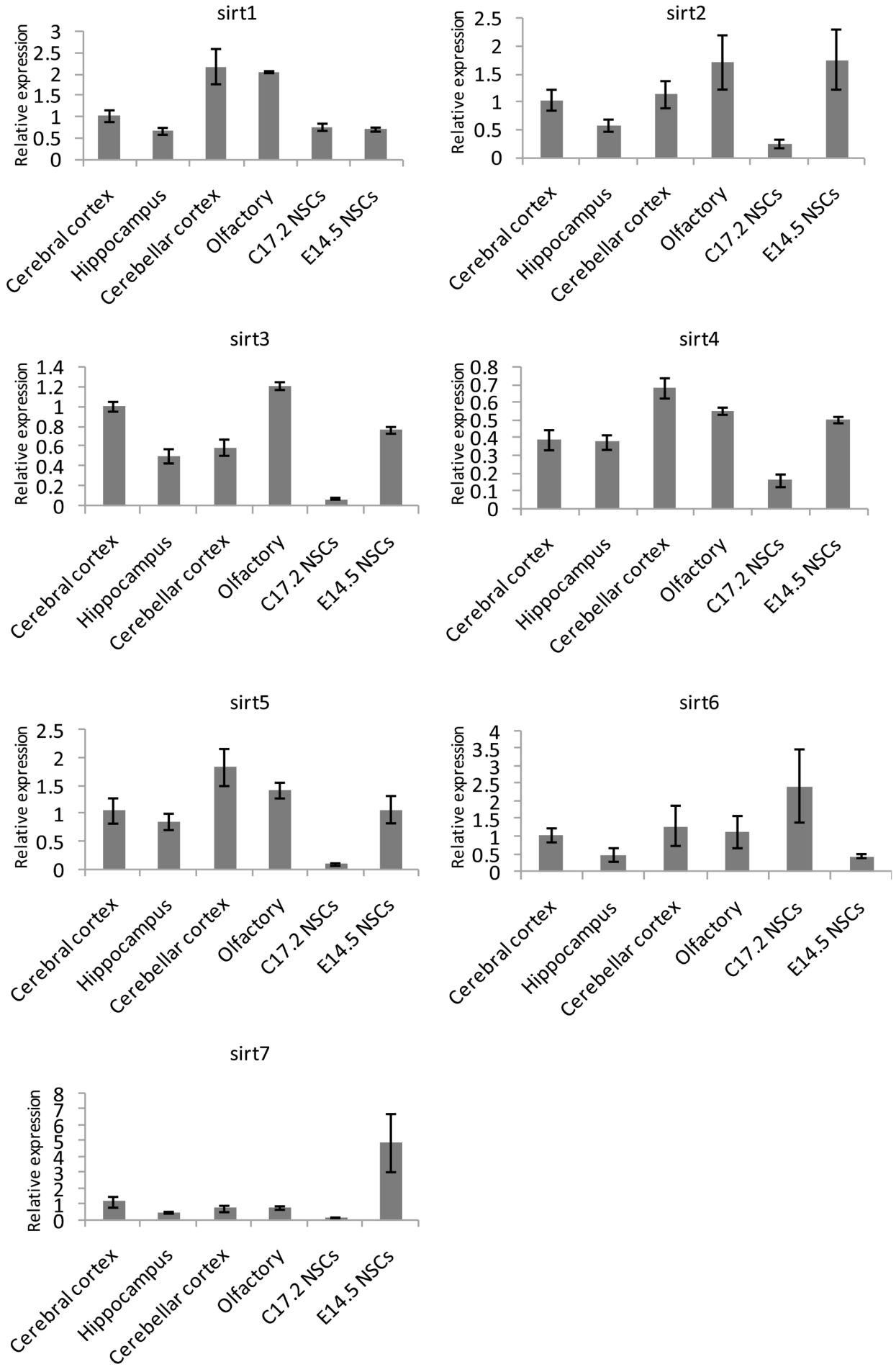

Figure 3. Detection of sirt1-sirt7 gene expression in different mouse brain tissues (cerebral cortex, hippocampus, cerebellar cortex, and olfactory bulb), C17.2 NSCs and E14.5 NSCs. (A) RNA from these tissues and cells were isolated and subjected to quantitative PCR analysis. Quantitative data (means±SEM) which are based on three independent experiments were normalized to β-actin.*$p<0.05$;**$p<0.01$.

and sirt7 expression was up-regulated, no statistical significance was detected whereas the expression did not changed in sirt2 and sirt4 (Fig. 4A, B). When treated with TSA (250nM), the mRNA expression was significantly down-regulated in sirt1 and sirt3, up-regulated in sirt2, sirt4, sirt6, and sirt7 significantly and unaffected in sirt5 (Fig. 4C, D).

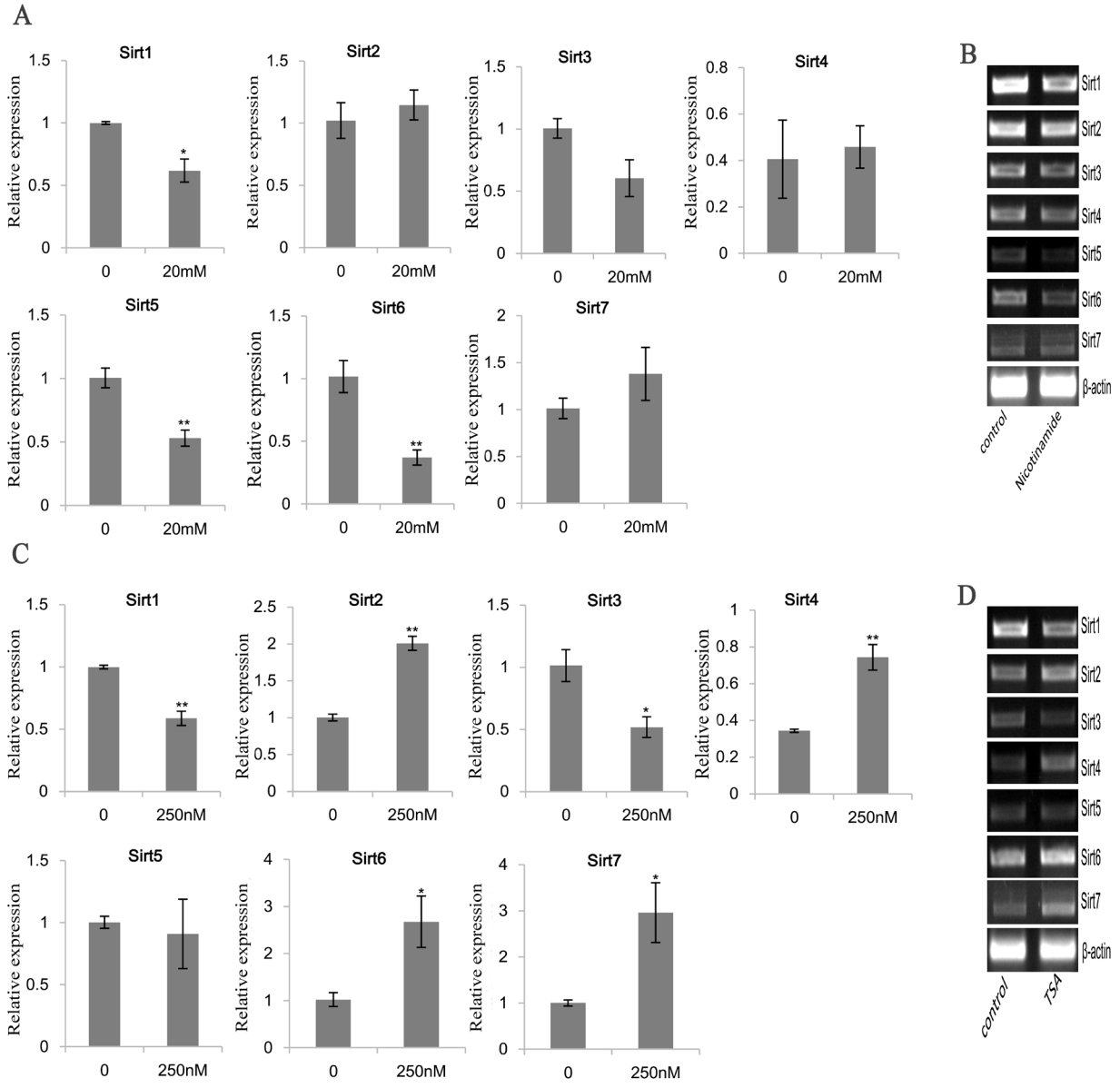

Figure 4. Effects of nicotinamide and TSA on the gene expression of sirt1-sirt7 in C17.2 NSCs. (A) C17.2 NSCs were cultured treated with nicotinamide (20mM), RNA from these cells was isolated and subjected to quantitative PCR analysis. Quantitative data (means±SEM) were normalized to β-actin, there data are based on three independent experiments.*p<0.05;**p<0.01. (B) Products of sirt1-sirt7 were observed by electrophoresed after exposue to nicotinamide. (C) C17.2 NSCs were cultured treated with TSA (250nM), RNA from these cells was isolated and subjected to quantitative PCR analysis. Quantitative data(means±SEM) which are based on three independent experiments were normalized to β-actin.*p<0.05;**p<0.01. (D) Products of sirt1-sirt7 were observed by electrophoresed after exposue to TSA.

DISCUSSION

In our present study, no significant difference at sirt1 mRNA expression level was found between C17.2 NSCs and E14.5 NSCs, suggesting that sirt1 may play a pivotal role in keeping normal NSCs status. Sirt1 is an important nutrient-sensitive coordinator for homeostatic process, stress-tolerance, and health span (20). Study showed that aging affected expression level of sirt1 in specific brain areas in mice as well as that gender also impacted transcription of sirt1 (21). Moreover, it also remains to be determined whether the downregulation of other sirtuins gene family affects the functions of C17.2 NSCs. In this study, transcriptional level of sirt2, sirt3, sirt4, sirt5 and sirt7 was significantly increased in E14.5 NSCs than C17.2 NSCs. Furthermore, similar to sirt1, sirt2 also can promote deacetylate of H3K56Ac (6). Report demonstrated that sirt2 maintains genome integrity and suppresses tumorigenesis through regulating APC/C Activity (19). Down-regulation of sirt2 can interfere genome integrity and cell proliferation. A recent study suggests that sirt3 activates the glutamate dehydrogenase (GDH) and isocitrate dehydrogenase (ICDH) 2 through deacetylate in the mitochondrial matrix further effects metabolism regulation (30). Sirt3 is involved in maintaining basal ATP levels and reversibly binds to and regulates the acetylation and activity of Com-

plex I in the electron-transport chain (3). Study demonstrated that sirt4 predominantly as an ADP-ribosyltransferase regulates GDH activity (12). But the acetylated substrates still were not identified for sirt4 (2). Sirt5 can deacetylate cytochrome c (cyt c) in the mitochondrial membrane space (IMS) and potentially regulate oxidative metabolism and apoptosis (30). Three sirtuins, sirt3, sirt4 and sirt5, are located in the mitochondria. Their up-regulation implied that a higher activity of sirtuin family is needed to maintain mitochondrial biology, energy production, metabolism, and apoptosis in E14.5 NSCs than in C17.2 NSCs. Studies showed that sirt7 is the only member of sirtuin family localized in nucleoli and no deacetylase or ADP-ribosyltransferase activity is detected. Moreover, sirt7 activity is essential for the resumption of rDNA transcription at the exit from mitosis (7,10). Up-regulation of sirt7 illustrated that sirt7 activity is needed to resume rDNA transcription in telophase in E14.5 NSCs. Conversely, down-expression of sirt7 may hinder resumption of rDNA, cell cycle and proliferation in C17.2 NSCs. In our study, sirt6 mRNA has a significant up-regulation in C17.2 NSCs. Some reports have demonstrated that sirt6 is a site-specific HDAC that plays important roles in genome stabilization, integrity of telomeric chromatin, aging-related gene expression, DNA repair, and maintaining metabolic homeostasis (25,31,32). Because of down-regulation in other sirtuin family member, the up-regulation of sirt6 is important to compensate sirtuin family member activity and further maintains basic cellular senescence, differentiation, life-span extension, and metabolism in C17.2 NSCs. Due to C17.2 NSCs is a cell line so the up-regulation of sirt6 can elucidate with better maintain genome stabilization and integrity of telomeric chromatin. Moreover, nutrient deprivation, fasting, and calorie-restricted diet promoted transcription level of sirt6 in HEK293 cells, mice, and rat (17). A large up-regulation of sirtuins gene family in four brain tissues suggests an important role in maintaining normal functions of differentiated neural-related cells. Surprisingly, olfactory bulb has a predominant upregulation of sirtuins, which demonstrated that olfactory bulb may present different transcription profiles of sirtuins mRNA distinguished from other brain tissues. mRNA transcription treated with TSA in C17.2 NSCs was similar to that in neuroblastoma cell line. Sirt2, sirt4, and sirt7 were upregulated whereas sirt1 was downregulation. Conversely, sirt6 was significant upregulation by TSA in C17.2 NSCs. In addition, no significant change was found in sirt5 when C17.2 NSCs exposed to TSA. Our results showed that both nicotinamide and TSA treatments affected gene expression of sirtuin family in C17.2 NSCs, and some changes were identical to those of previous reports (15), others refered exclusively to C17.2 NSCs. Interestingly, in contrast to TSA, nicotinamide induced downregulation of sirt5 in C17.2 NSCs, suggesting that transcription regulation of sirt5 might through class III HDAC instead of class I and II HDAC. Conversely, TSA treatment promoted upregulation of sirt2 and sirt4, illustrating that gene expression of sirt2 and sirt4 mainly through class I and II HDAC.

In summary, our studies indicated that transcription levels of sirtuin family in C17.2 neural stem cells differ from primary neural stem cells and adult mouse brain, moreover, the transcriptional levels of sirtuin family can be selectively regulated by different histone acetylation in neural development process.

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