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Effect of Histone Deacetylase Inhibitors on Differentiation of Human Bone Marrow-derived Stem Cells Into Neuron-like Cells

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Abstract

Mesenchymal stem cells (MSCs) are known to differentiate into multiple lineages, making neurogenic differentiation an important target in the clinical field. In the present study, we induced the neurogenic differentiation of cells using histone deacetylase (HDAC) inhibitors and studied their mechanisms for further differentiation *in vitro*. We treated cells with the HDAC inhibitors, MS-275 and NaB; and found that the cells had neuron-like features such as distinct bipolar or multipolar morphologies with branched processes. The mRNA expressions encoding for *NEFL*, *MAP2*, *TUJ1*, *OLIG2*, and *SYT* was significantly increased following HDAC inhibitors treatment compared to without HDAC inhibitors; high protein levels of MAP2 and Tuj1 were detected by immunofluorescence staining. We examined the mechanisms of differentiation and found that the Wnt signaling pathway and downstream mitogen-activate protein kinase were involved in neurogenic differentiation of MSCs. Importantly, Wnt4, Wnt5a/b, and Wnt11 protein levels were highly increased after treatment with NaB; signals were activated through the regulation of Dvl2 and Dvl3. Interestingly, NaB treatment increased the levels of JNK and upregulated JNK phosphorylation. After MS-275 treatment, Wnt protein levels were decreased and GSK-3β was phosphorylated. In this cell, HDAC inhibitors controlled the non-canonical Wnt expression by activating JNK phosphorylation and the canonical Wnt signaling by targeting GSK-3β.

Keywords : Glycogen Synthase Kinase 3, Histone Deacetylase Inhibitors, Mesenchymal Stromal Cells, Wnt Signaling Pathway, Cell Differentiation

1. Introduction

Mesenchymal stem cells (MSCs) are used for regeneration or repair because of their capacities for self-renewal and multilineage differentiation[1]. Human bone marrow-derived MSCs (hBM-MSCs) have the advantages of availability, easy culture expansion, and low immunogenic properties, and thus are commonly used in clinical applications[2]. Although numerous studies have examined the control of hBM-MSCs differentiation into neuron-like cells by modulating cell signaling pathways, the regulatory molecules and molecular mechanisms involved in differentiation remain unclear.

Histones, which exist in two antagonist forms, acetylated or deacetylated, play important roles in gene regulation. Histone deacetylation results in wrapping DNA more tightly following removing acetyl groups on a histone. Thus, histone deacetylases (HDACs) are important to stabilize nucleosomal structures and to repress transcription and also known to regulate various biological processes, for example, proliferation, differentiation, and developmental progress [3-5]. In general, small-molecule compounds can be synthesized with a high quantity, stability, and purity; and show low risks. Additionally, these molecules can be used as differentiation-inducing factors into neuron-like cells from hBM-MSCs[6]. HDAC inhibitors are natural or synthetic small molecules with effects on cell homeostasis, gene expression, cell cycle progression, cell migration, terminal differentiation, and cell death[7].

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HDACs are grouped into four main classes and restrained by broad or specific inhibitors. MS-275 is a class I HDAC1 and HDAC3 inhibitor that modulates embryonic stem cell differentiation[8]. MS-275 regulated the excitatory synaptic structure and showed to play an important modulator of depression and anxiety-like behavior[9]. Sodium butyrate (NaB) is a class I, IIa, and IV HDAC inhibitor; which functions in proliferation inhibition, differentiation induction, and gene expression induction or repression[10]. NaB promotes rat BM-MSCs differentiation into smooth muscle cells by regulating intracellular calcium levels[11]. In stem cells, VPA, which is one of the HDAC inhibitors, stimulates neurogenesis by up-regulating NeuroD[12,13] and inducing MSCs to differentiate into neuron-like cells by activating the Wnt signaling pathway[14,15]. However, the effect of MS-275 and NaB on neuronal differentiation in stem cells is not studied yet.

In our experiments, we investigated how different combinations of neurogenic supplementary factors and HDAC inhibitors affects the differentiation of neuron-like cells in hBM-MSCs and regulates downstream signaling pathways, particularly, Wnt signaling. Moreover, we demonstrated that HDAC inhibitors stimulate the neurogenic differentiation of hBM-MSCs by controlling phosphorylated mitogen-activate protein (MAP) kinase signals.

2. Methods

2.1. Cell lines and reagents

We used two different HDAC inhibitors; MS-275 (500 nM) and NaB (10 μM), following our previously published methods[15].

In addition, hBM-MSCs were cultured and differentiated into NI-hBM-MSCs following our previously published methods[1,2,16,17]. Cells were obtained according to the guidelines established by the Ethics Committee at the Chonnam National University Medical School (IRB: I-2009-03-016). The cells were grown as adherent cultures in supplemented with 10% fetal bovine serum (Hyclone), 1% penicillin-streptomycin (Gibco BRL, Grand Island, NY, USA), and 0.2% amphotericin B (Gibco BRL) in a 37°C humidified incubator with 5% CO_2. To induce neural differentiation, the cells were maintained in 100 ng/mL basic fibroblast growth factor (Invitrogen Co., Carlsbad, CA, USA), for 7 days, and then incubated in 10 μM forskolin (Sigma Chemical Co.) for an additional 7 days. The HDAC inhibitors were added individually during the 14-day neural induction.

2.2. Immunocytochemistry

The immunocytochemical procedure was modified from our previously published study[1,18]. Cells were fixed for 15 min with chilled-methanol (Burdick & Jackson, Muskegon, MI, USA), and blocked for 20 min with 10% normal goat serum in 0.5% Triton X-100 (Sigma Chemical Co.). Anti-MAP2 and anti-Tuj1 were then incubated with the cells overnight at 4°C. All primary antibodies were purchased from Cell Signaling Technology Inc. and used as diluted 1:300. After washing, the Alexa 488-conjugated goat anti-mouse antibody (1:300, Molecular probes, Invitrogen Co., CA, USA) was used as a secondary antibody and incubated at room temperature for 1 h. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI; Thermo Fisher Scientific, Waltham, MA, USA) to enable cell counting. Cells were observed using a Zeiss LSM510 confocal microscope (Carl Zeiss, Jena, Germany). Experiments were performed in triplicate, and the percentage of positive cells was randomly calculated. For quantitative analysis, the number of positive cells was counted in each acquired image by ImageJ 1.61 software (NIH, Bethesda, MD, USA), and the ratio of the number of positive cells to the number of nuclei was analyzed for each antigen.

2.3. Reverse Transcriptase-polymerase Chain Reaction (RT-PCR) Analysis

Total RNA was prepared in TriZol (Molecular Research Center, Inc., Cincinnati, OH, USA) and cDNA was synthesized; according to the manufacturer's instructions and modified from our previously reported study[15]. The cDNA was amplified by 35 cycles of 94°C for 1 min, appropriate annealing temperatures for each primer for 1 min, and 72°C for 1 min, using the Ex-Taq polymerase (Takara Bio, Inc., Shiga, Japan) on a PCR machine (Takara Bio, Inc.). The forward and reverse PCR oligonucleotide primers used to amplify the cDNA are listed in Table 1 (Bioneer Co., Daejeon, Korea). RT-PCR products were verified by electrophoresis on 2% agarose gels (Sigma Chemical Co.) The amount of cDNA was normalized based on

Table 1. Sequences of PCR primers

Gene	Forward (5'-3')/ Reverse (5'-3')
NEUROD1	ACAGCTCCCATGTCTTCCAC
	AAGATTGATCCGTGGCTTTG
NEFL	TCCTACTACACCAGCCATGT
	TCCCCAGCACCTTCAACTTT
NEFM	TGGGAAATGGCTCGTCATTT
	CTTACTGGAAGCGGCCAATT
MAP2	TGCCATCTTGGTGCCGA
	CTTGACATTACCACCTCCAGGT
TUJ1	CATGGATGTCCGCTCAG
	CAGGCAGTCGCAGTTTTCAC
SYT	CGCAAACTGGGCAAACGCTA
	GCAACCCTCGTGGGCCTC
GAP43	TCCTGAGCCTGTCTCTCCCT
	GCCACACTGTTTGACTTGGG
CNP	GGCCACGCTGCTAGAGTGCAAGAC
	GGTACTGGTACTGGTCGGCCATTT
OLIG2	AAATCGCATCCAGATTTTC
	CACTGCCTCCTAGCTTGTC
GAPDH	CATGACCACAGTCCATGCCATCACT
	TGAGGTCCACCACCTGTTGCTGTA

the level of the ubiquitously expressed glyceraldehyde 3-phosphate (*GAPDH*) to analyze the relative expression of mRNAs[1,16,17]. The RT-PCR assay was repeated five times independently using four different donor cells.

2.4. Quantitative RT-PCR

For quantitative RT-PCR analyses, cDNA was synthesized and prepared following our previous reports[16,17]. Quantitative RT-PCR was performed with SYBR Green Premix Ex Taq (Takara Bio Inc.) and Thermal Cycler Dice Real Time System (Takara Bio Inc.). Each sample was analysed in four replicate reactions of 20 μL.

2.5. Western Blot Assay

The cells were cultured and Wnt-related signaling pathways were examined by western blot analysis. As described in our previous studies[16,17], the cells were lysed in extraction buffer and an equal amount (20 μg) of protein for each sample was resolved by 8-12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The transferred membranes were incubated with specific antibodies for Wnt1, Wnt4, Wnt11, phosphor-JNK, JNK, GAPDH (Santa Cruz Biotechnology, Dallas, TX, USA), Wnt5a/b, Naked1, Naked2, Dvl2, Dvl3, β-catenin, and phosphor-GSK-3β, GSK-3β (Cell Signaling Technology). The signals were detected with enhanced chemiluminescence reagents (Millipore Corporation, Billerica, MA, USA) using an LAS 4000 luminescent image analyzer (GE Healthcare, Little Chalfont, UK). The density was quantified using ImageJ software. Western blot analysis was repeated five times independently using four different donor cells.

2.6. Statistics

The mRNA and protein expression levels were quantified by measuring the optical density of each band by computer-assisted densitometry (NIH Image analysis program, version 1.61). One-way analysis of variance (Bonferroni *post-hoc* comparison) was performed to analyze differences between groups, with $*p < 0.05$ and $**p < 0.01$ considered to indicate statistical significance. All values are expressed as the mean ± standard error of the mean.

3. Results

3.1. HDAC Inhibitors Enhance Neuron-like Cell Differentiation of hBM-MSCs in Vitro

To investigate the impact of HDAC inhibitors on neuron-like cell differentiation of hBM-MSCs, we selected MS-275 and NaB and studied their abilities to induce stem cell differentiation. Primary human BM-MSCs were isolated from human bone marrow and cultured until they uniform exhibited typical fibroblast-like morphology. For neurogenic differentiation, cells; obtained from human bone marrow and characterized as MSCs; were incubated in the presence of the HDAC inhibitors; MS-275 (500 nM) or NaB (10 μM). All neurogenic-differentiated hBM-MSCs, including those treated with MS-275 or NaB, exhibited a neuronal morphology, while primary hBM-MSCs showed a fibroblast-like feature with a flattened and spindle shape. After treating the cells with combinations of MS-275 or NaB, the neurite number on the cells was increased and branched processes were elongated. The cells were visualized by immunofluorescence staining of the neuronal markers; microtubule associated protein-2

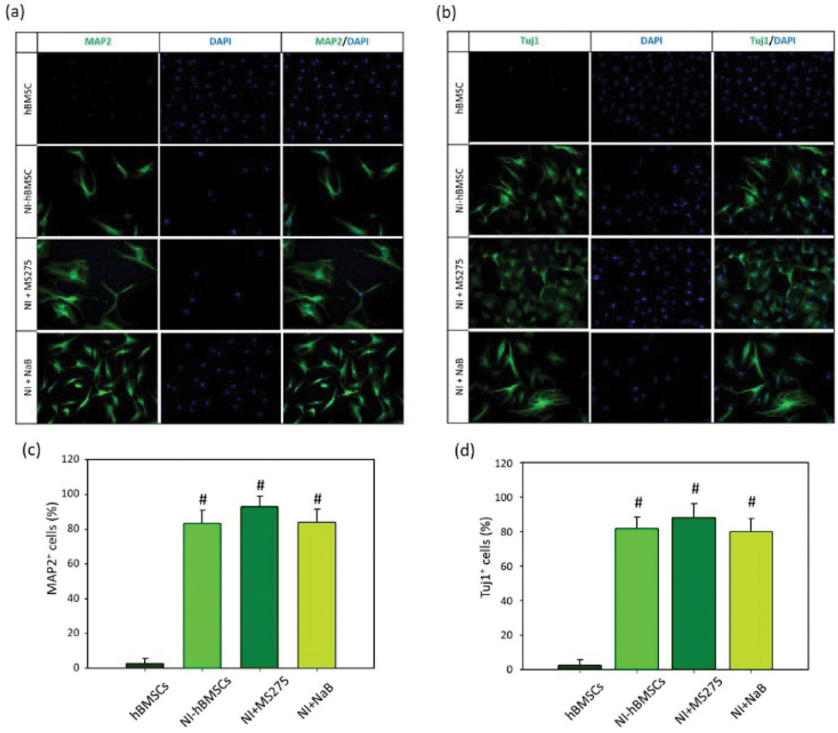

Fig. 1. The fluorescent immunocytochemistry of hBM-MSCs. (a and b) Human BM-MSCs were induced to differentiate into neural cells in the presence of bFGF and forskolin over 2 weeks. Following MS-275 or NaB treatment, the typical fibroblast-like morphology were changed to bipolar of multipolar ones with branched processes. Fluorescent data revealed that the expression of MAP2 and Tuj1 following treatment with the MS-275 or NaB in NI-hBM-MSCs was higher than without HDAC inhibitors. (c and d) The number of positive cells was counted and the ratio of positive cells to nuclei was analyzed for the antigen. #$p<0.01$ compared with the ratio from primary hBM-MSCs.

(MAP2) and neuron-specific class III β-tubulin (Tuj1) (Fig. 1). The cells showed distinct bipolar or multipolar morphologies with branched processes, which were visualized by immunofluorescence staining of the somatodendritic marker; MAP2 (Fig. 1a). Additionally, the expression of Tuj1, which indicates differentiated neurons and is an important neuronal marker, was highly increased after treatment with the HDAC inhibitors (Fig. 1b and 1d). Primary hBM-MSCs did not express MAP2; however, MAP2 was highly expressed after neurogenic differentiation (Fig. 1c).

RT-PCR analysis was used to monitor the extent of neurogenic differentiation in mRNA levels of hBM-MSCs. After treatment with MS-275 or NaB, the levels of mRNAs encoding for the neuronal markers neurofilament-L (*NEFL*), and *TUJ1* were increased; compared to the levels in NI-hBM-MSCs (Fig. 2). Interestingly, *MAP2* and oligodendrocyte transcription factor (*OLIG2*) levels were highly expressed after treatment with MS-275. However, other neuronal markers such as neurogenic differentiation 1 (*NEUROD1*) and neurofilament-M (*NEFM*) did not change. Additionally, the expression of synaptotagamin (*SYT)*, 2'3'-cyclic nucleotide 3-phosphodiesterase (*CNP*), and growth associated protein 43 (*GAP43*) did not change.

To confirm the mRNA expression of neuron-specific markers, we performed quantitative RT-PCR analysis of neuronal marker genes. Interestingly, *NEFL* and *TUJ1* gene expressions were strongly expressed with NaB and *MAP2* and *OLIG2* were highly increased after treatment of MS-275, significantly (Fig. 3).

3.2. HDAC Inhibitors Regulate Wnt Pathway and Downstream JNK Signaling

Following our previous reports, Wnt signaling is one of the important pathways in the neurogenic differenti-

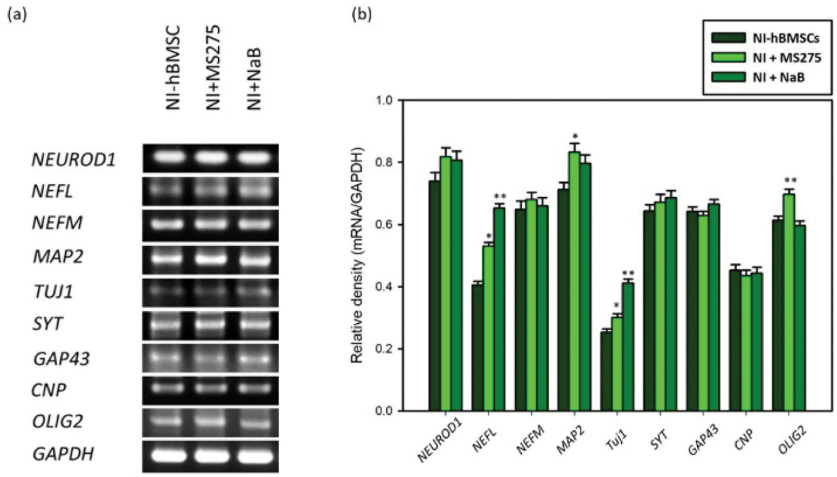

Fig. 2. RT-PCR analysis of neurogenic gene expressions. We establish the mRNA expression of the neurogenic differentiation-related genes following treatment of NI-hBM-MSCs (a) and quantify the results (b). The expression of diverse neurogenic markers was increased following NI-hADSC treatment with the MS-275 or NaB. *GAPDH* was used as a control. The RT-PCR assay was repeated five times independently in four different donor cells, and the representative data are shown. The expression level of each gene was normalized to GAPDH. *p<0.05, **p<0.01 compared to NI-hBM-MSCs. NaB, sodium butyrate; *NEUROD1*, neurogenic differentiation 1; *NEFL*, neurofilament-L; *NEFM*, neurofilament-M; *MAP2*, microtubule associated protein-2; *TUJ1*, neuron-specific class Ⅲ β-tubulin; *SYT*, synaptotagmin; *GAP43*, growth associated protein 43; *CNP*, 2'3'-cyclic nucleotide 3'-phosphodiesterase; *OLIG2*, oligodendrocyte transcription factor; *GAPDH*, glyceraldehyde 3-phosphate; RT-PCR, Reverse transcriptase-polymerase chain reaction.

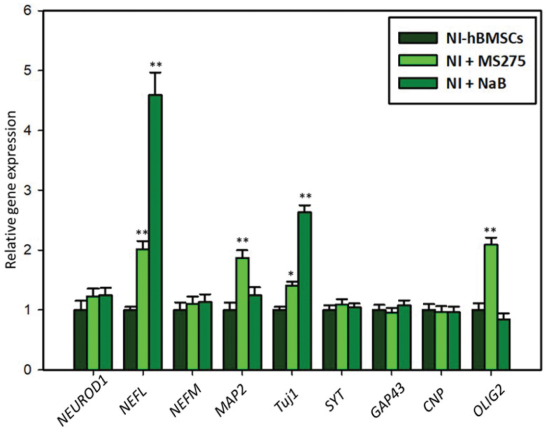

Fig. 3. Quantitative RT-PCR analysis of neurogenic gene expression. Quantitative RT-PCR was performed to confirm the RT-PCR assay. This study was repeated four times independently of different cells and the representative data are shown. The intensity of each gene was normalized to *GAPDH* and compared with the NI-hBM-MSCs.

ation and HDAC inhibitor treatment can be differentiated into neuron-like cells in adipose tissue-derived MSCs [15-18]. Until now, effect of HDAC inhibitors through Wnt pathway in human BM-MSCs is not clear. To investigate the Wnt signaling pathway with HDAC inhibitors for neurogenic differentiation in hBM-MSCs, protein expression was studied by western blot analysis. As shown in Fig. 4a and 4b, HDAC inhibitors up-regulated the Wnt4 level compared to that without. Interestingly, the levels of Wnt5a/b and Wnt11 were highly increased by NaB and decreased by MS-275. The levels of both Naked1 and Naked2, which regulate the Wnt signaling pathway by binding to Dishevelled proteins and activating the planar cell polarity pathway, were expressed oppositely following treatment. Additionally, Dvl2 level was increased by NaB treatment compared to in NI-hBM-MSCs. These results demonstrate that HDAC inhibitors can induce neurogenic differentiation by activating the Wnt signaling pathway in MSCs.

To further study of Wnt-related downstream signaling in neurogenic differentiation through HDAC inhibitors, we demonstrated the signal transduction pathways, such as that involving MAP kinase, by western blot analysis. The level of phosphorylated JNK was highly increased by NaB treatment (Fig. 4c and 4d). In the

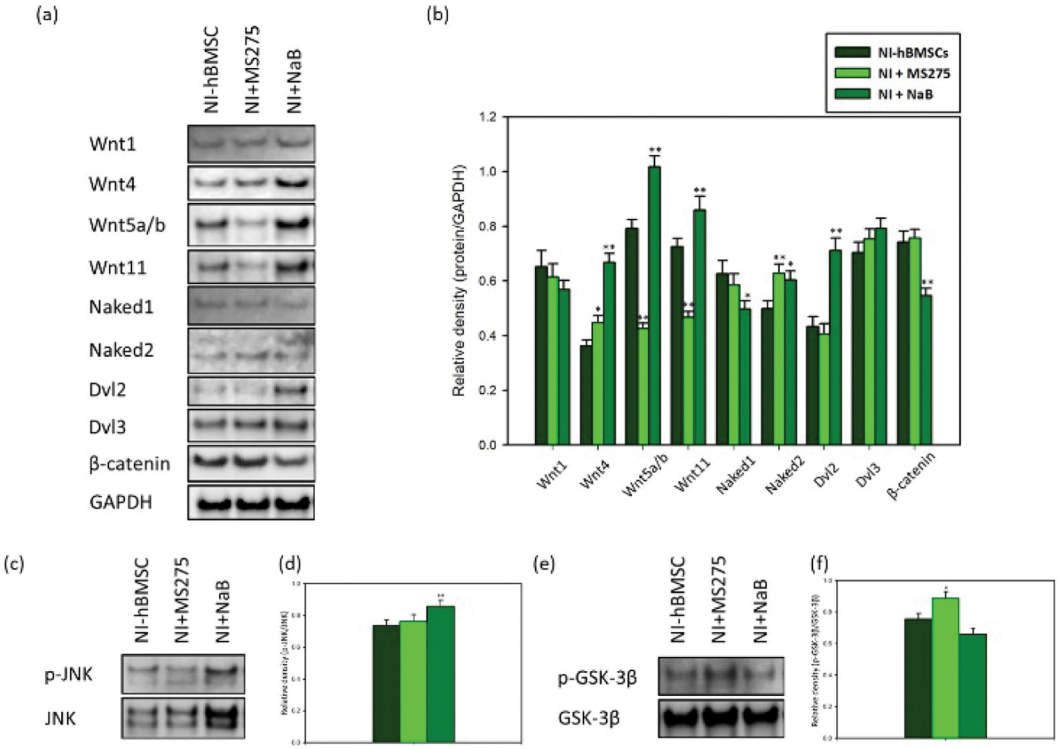

Fig. 4. Expression of the Wnt signaling-related proteins. To evaluate Wnt signaling following NI-hBM-MSC treatment with the MS-275 or NaB, the protein expressions were determined by western blot analysis (a) and the results were quantified (b). The expression of phosphor-JNK, which is one of the downstream non-canonical pathway, is upregulated with NaB (c and d). In addition, GSK-3β, which is major signaling of canonical pathway, was phosphorylated with MS-275 treatment (e and f). Three independent biological replicates were performed, and at least five technical replicates were performed for each biological replicate.

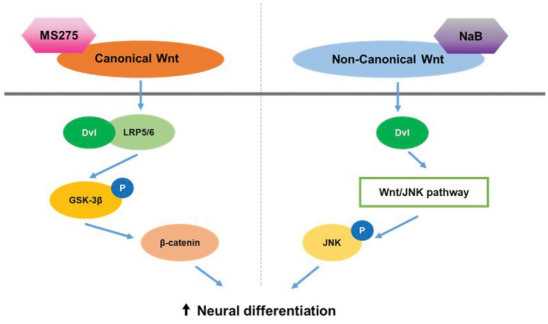

Fig. 5. Wnt signaling with MS-275 and NaB treatment during neurogenic differentiation: a simplified view. In canonical Wnt pathway, Wnt signals through activation of LRP5/6 and Dvl complex, phosphorylation of GSK-3β, and accumulation of β-catenin by MS-275. A non-canonical Wnt enhance the activation of the Wnt/JNK pathway by NaB and finally activates the neurogenic differentiation of NI-hBM-MSCs.

canonical Wnt pathway, the Wnt/Fzd complex functions by activating Dvl to inhibit the degradation of β-catenin by GSK-3β. The non-canonical Wnt pathway has mainly two different manners: 1) by increasing intracellular calcium and protein kinase C levels; and 2) by activating c-Jun N-terminal kinase (JNK). Protein expression of JNK and phosphor-JNK, which are key molecules in the non-canonical Wnt signaling pathway, was highly increased with HDAC inhibitor treatment compared to those in NI-hBM-MSCs. Interestingly, the GSK-3β and phosphor-GSK-3β protein levels were increased in MS-275-treated NI-hBM-MSCs (Fig. 4e and 4f). These data indicate that HDACs are important modulators that activate both the canonical and non-canonical Wnt pathways and regulate JNK and GSK-3β expression in the neurogenic differentiation of hBM-MSCs (Fig. 5).

4. Discussion

HDACs exhibit a highly pleiotropic ability in stem cell proliferation and differentiation including by acting on MSC differentiation. However, the role of the HDAC inhibitors in the neurogenic differentiation of human BM-MSCs had not been examined. Our findings demonstrate that the HDAC inhibitors participate in neurogenic differentiation by regulating both canonical and non-canonical Wnt pathways.

In this study, MS-275 and NaB were tested using our neurogenic differentiation protocol. The phenotypes of NI-hBM-MSCs treated with HDAC inhibitors exhibited neuron-like features and MAP2- and Tuj1-positive cells were increased. Valproic acid (VPA), which is one of the HDAC inhibitors, treatment increased MAP2- and Tuj1-positive neuronal cells and enhanced superficial cortical neurons in mouse embryonic stem cells[19]. Other group also reported that VPA-treated cells highly expressed MAP2 and Tuj1 according to immunofluorescent analysis in human placental MSCs[20].

Our previous study showed that MS-275, NaB, TSA, or VPA stimulates the neurogenic differentiation of adipose tissue-derived MSCs [15]. Other recent studies reported that HDAC inhibitors enhance stem cell differentiation, particularly neuron-like cells[3,6,13,14]. After treatment with HDAC inhibitors, neuronal marker genes were increased compared to in NI-hBM-MSCs (Fig. 2 and Fig. 3). Interestingly, MS-275, an HDAC inhibitor used in both this study and our previously published study[15], has different functions in MSCs of different origins. Accordingly, the expression of neuronal marker genes was increased in adipose-derived MSCs [15]; however, these levels were not changed or decreased in bone marrow-derived MSCs.

The Wnt protein plays a major role in various processes including proliferation, differentiation, and fate decision[14,21]. Recently, some studies showed that the canonical Wnt/β-catenin pathway supports pluripotency, whereas other studies reported a role in cell differentiation. We predicted that canonical or non-canonical Wnt pathways have different roles in mesenchymal stem cell types of different origins, such as bone marrow-derived stem cells or adipose tissue-derived stem cells. In this study, the non-canonical Wnt pathway was involved in regulation by upregulating JNK expression for neurogenic differentiation of hBM-MSCs following NaB treatment (Fig. 4). In hADSCs, several HDAC inhibitors including NaB can enhance the neurogenic differentiation through non-canonical Wnt pathway[15]. Additionally, the canonical Wnt pathway, which is regulated via GSK-3β, is expressed in hBM-MSCs following MS-275 treatment; otherwise canonical Wnt is not expressed in hADSCs according to our previous studies[15,18]. These data demonstrate that both canonical and non-canonical Wnt pathways are involved in the neurogenic differentiation of hBM-MSCs. Stem cells is known to retain the ability to differentiate into mature cells, however, this study suggests that the molecular mechanism of stem cell differentiation could be differ from their origin.

Our data showed that MS-275 and NaB promote stem cell differentiation into neurons through the Wnt signaling pathway (Fig. 5). Additionally, the different effects of HDAC inhibitors on neurogenic differentiation depends on the origin of the cell type. Our findings provide novel information regarding stem cell responses to HDAC inhibitors and a potential therapy for clinical application. Further studies are needed to link genetic and developmental studies using Wnt agonists and antagonists for neurogenic differentiation of MSCs.

5. Conclusions

Overall, our study revealed that HDACs are important modulators that activate both the canonical and non-canonical Wnt pathways and regulate JNK and GSK-3β expression in the neurogenic differentiation of hBM-MSCs.

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References

[1] S. Jang, H.H. Cho, Y.B. Cho, J.S. Park, H.S. Jeong. Functional neural differentiation of human adipose

tissue-derived stem cells using bFGF and forskolin. *BMC Cell Biol*. 2010;11:25.

[2] S. Jang, H.H. Cho, S.H. Kim, K.H. Lee, J.Y. Jun, J.S. Park, H.S. Jeong, Y.B. Cho. Neural-induced human mesenchymal stem cells promote cochlear cell regeneration in deaf Guinea pigs. *Clin Exp Otorhinolaryngol*. 2015;8:83-91.

[3] A. Fila-Danilow, P. Borkowska, M. Paul-Samojedny, M. Kowalczyk, J. Kowalski. The influence of TSA and VPA on the in vitro differentiation of bone marrow mesenchymal stem cells into neuronal lineage cells: Gene expression studies. *Postepy Hig Med Dosw (Online)*. 2017;71:236-242.

[4] C.D. Zimberlin, C. Lancini, R. Sno, S.L. RosekransL, C.M. McLean, H. Vlaming, G.R. van den Brink, M. Bots, J.P. Medema, J.H. Dannenberg. HDAC1 and HDAC2 collectively regulate intestinal stem cell homeostasis. *FASEB J*. 2015;29:2070-2080.

[5] J. Yang, Y. Tang, H. Liu, F. Guo, J. Ni, W. Le. Suppression of histone deacetylation promotes the differentiation of human pluripotent stem cells towards neural progenitor cells. *BMC Biol.* 2014;12:95.

[6] Y. Kondo, T. Iwao, S. Yoshihashi, K. Mimori, R. Ogihara, K. Nagata, K. Kurose, M. Saito, T. Niwa, T. Suzuki, N. Miyata, S. Ohmori, K. Nakamura, T. Matsunaga. Histone deacetylase inhibitor valproic acid promotes the differentiation of human induced pluripotent stem cells into hepatocyte-like cells. *PLoS One.* 2014;9:e104010.

[7] A. Kretsovali, C. Hadjimichael, N. Charmpilas. Histone deacetylase inhibitors in cell pluripotency, differentiation, and reprogramming. *Stem Cells Int.* 2012;2012:184154.

[8] G. Franci, L. Casalino, F. Petraglia, M. Miceli, R. Menafra, B. Radic, V. Tarallo, M. Vitale, M. Scarfo, G. Pocsfalvi, A. Baldi, C. Ambrosino, N. Zambrano, E. Patriarca, S. De Falco, G. Minchiotti, H.G. Stunnenberg, L. Altucci. The class I-specific HDAC inhibitor MS-275 modulates the differentiation potential of mouse embryonic stem cells. *Biol Open.* 2013;2:1070-1077.

[9] S.A. Golden, D.J. Christoffel, M. Heshmati, G.E. Hodes, J. Magida, K. Davis, M.E. Cahill, C. Dias, E. Ribeiro, J.L. Ables, P.J. Kennedy, A.J. Robison, J. Gonzalez-Maeso, R.L. Neve, G. Turecki, S. Ghose, C.A. Tamminga, S.J. Russo. Epigenetic regulation of RAC1 induces synaptic remodeling in stress disorders and depression. *Nat Med.* 2013; 19:337-344.

[10] T. Lilja, N. Heldring, O. Hermanson. Like a rolling histone: epigenetic regulation of neural stem cells and brain development by factors controlling histone acetylation and methylation. *Biochim Biophys Acta.* 2013;1830:2354-2360.

[11] J. Liu, Y. Wang, Y. Wu, B. Ni, Z. Liang. Sodium butyrate promotes the differentiation of rat bone marrow mesenchymal stem cells to smooth muscle cells through histone acetylation. *PLoS One*. 2014; 9:e116183.

[12] S.G. Jeong, T. Ohn, S.H. Kim, G.W. Cho. Valproic acid promotes neuronal differentiation by induction of neuroprogenitors in human bone-marrow mesenchymal stromal cells. *Neurosci Lett.* 2013;554:22-27.

[13] T. Okubo, D. Hayashi, T. Yaguchi, Y. Fujita, M. Sakaue, T. Suzuki, A. Tsukamoto, O. Murayama, J. Lynch, Y. Miyazaki, K. Tanaka, T. Takizawa. Differentiation of rat adipose tissue-derived stem cells into neuron-like cells by valproic acid, a histone deacetylase inhibitor. *Exp Anim*. 2016;65:45-51.

[14] L. Wang, Y. Liu, S. Li, Z.Y. Long, Y.M. Wu. Wnt signaling pathway participates in valproic acid-induced neuronal differentiation of neural stem cells. *Int J Clin Exp Pathol.* 2015;8:578-585.

[15] S. Jang, H.S. Jeong. Histone deacetylase inhibition-mediated neuronal differentiation via the Wnt signaling pathway in human adipose tissue-derived mesenchymal stem cells. *Neurosci Lett*. 2018; 668:24-30.

[16] S. Jang, H.H. Cho, J.S. Park, H.S. Jeong. Non-canonical Wnt mediated neurogenic differentiation of human bone marrow-derived mesenchymal stem cells. *Neurosci Lett*. 2017;660:68-73.

[17] S. Jang, J.S. Park, H.S. Jeong. Neural Differentiation of Human Adipose Tissue-Derived Stem Cells Involves Activation of the Wnt5a/JNK Signalling. *Stem Cells Int.* 2015;2015:178618.

[18] S. Jang, H.S. Jeong. Data for the effect of histone deacetylase inhibitors on voltage- and ligand-gated ion channel gene expression in neurogenic induced-human adipose tissue-derived mesenchymal stem cells. *Data Brief.* 2018;17:1314-1319.

[19] B. Juliandi, M. Abematsu, T. Sanosaka, K. Tsujimura, A. Smith, K. Nakashima. Induction of superficial cortical layer neurons from mouse embryonic stem cells by valproic acid. *Neurosci Res.* 2012;72:23-31.

[20] M. Talwadekar, S. Fernandes, V. Kale, L. Limaye.

Valproic acid enhances the neural differentiation of human placenta derived-mesenchymal stem cells in vitro. *J Tissue Eng Regen Med*. 2016; doi:10.1002/term.2219.

[21] A. Bahrami, F. Amerizadeh, S. ShahidSales, M. Khazaei, M. Ghayour-Mobarhan, H.R. Sadeghnia, M. Maftouh, S.M. Hassanian, A. Avan. Therapeutic Potential of Targeting Wnt/beta-Catenin Pathway in Treatment of Colorectal Cancer: Rational and Progress. *J Cell Biochem*. 2017;118:1979-1983.