THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 284, NO. 5, pp. 2891–2901, January 30, 2009

Reversine Increases the Plasticity of Lineage-committed Cells toward Neuroectodermal Lineage*[S]

Received for publication, May 28, 2008, and in revised form, November 17, 2008 Published, JBC Papers in Press, November 17, 2008, DOI 10.1074/jbc.M804055200

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Functional dedifferentiation of lineage-committed cells toward pluripotency may have a great potential in regenerative medicine. Reversine has been shown to induce dedifferentiation of multiple terminally differentiated mesodermal origin cells, which are capable of being directed to differentiate into other cell types within mesodermal lineages. However, the possibilities of these cells to give rise to other lineages have not been examined. Here we show that large scale gene expression profiling of reversine-treated C2C12 myoblasts identifies a subset of up-regulated genes involved in specification of neuroectodermal as well as mesodermal lineages. Reversine treatment leads to up-regulation of priming genes of neuroectodermal lineages, such as *Ngn2*, *Nts*, *Irx3*, *Pax7*, *Hes1*, and *Hes6*, through active histone modifications in the promoter regions of these genes. Additionally, reversine increases the expression of markers for other cell types of mesodermal lineages, Ogn and apoE, via inducing active histone modifications, while down-regulating the myogenic basic helix-loop-helix factor, MyoD, via repressive histone modifications. Consistent with up-regulation of these genes, reversine-treated C2C12 myoblasts redifferentiate into neural as well as mesodermal lineages, under appropriate stimuli. Taken together, these results indicate that reversine induces a multipotency of C2C12 myoblasts via inducing a specific combination of active histone modifications. Collectively, our findings provide a mechanistic rationale for the application of reversine to dedifferentiation of somatic cells.

Stem cells that possess an unlimited potential for self-renewal and the ability to differentiate into various specialized cells in response to appropriate signals can be classified into embryonic and tissue-specific stem cells. Embryonic stem (ES)[3] cells are derived from the inner cell mass of the mammalian blastocysts and can differentiate into all kinds of somatic cell types. In contrast, tissue-specific adult stem cells are found in differentiated tissues as unspecialized cells and can self-renew and differentiate into specialized cell types of the tissue in which they reside (1–4). The characteristics of stem cells attract great attention for their potential therapeutic use for degenerative diseases, including type I diabetes, muscular dystrophies, cancers, and neurodegenerative and cardiovascular diseases. However, the applications of stem cells for cell replacement therapy have a number of problems such as the limited propagation capacity and the restricted differentiation of stem cells, as well as allograft rejection (5).

Dedifferentiation of adult differentiated cells into multipotent progenitors could be a very attractive alternative to overcome many of these obstacles (6). Although somatic cells are thought to be stably committed to their fate, emerging evidence indicates that dedifferentiation events can occur through nuclear transfer (7), cell fusion (8–10), ectopic gene expression (11), and exogenous factors (12). For example, nuclear transfer of adult somatic cell into unfertilized eggs has been shown to dedifferentiate the somatic cells into pluripotent ES cells and give birth to cloned animals. Similarly, fusion of neuronal progenitor cells or bone marrow-derived cells with ES cells results in hybrids that express markers of pluripotency and contribute to chimeras. Similar observations resulted from fusing human fibroblasts with ES cells. Fusion of embryonic carcinoma (EC) cells with T-lymphoma cells also promotes the formation of colonies expressing pluripotent cell transcripts from the lymphoma genome. Thus, components of pluripotent ES or EC cells have the potential of eliciting reprogramming events in a somatic genome. Recently, a major breakthrough has been reported whereby the combined expression of four transcription factors Oct4, Sox2, c-Myc, and Klf4 in fibroblasts is sufficient to induce fibroblasts to become pluripotent stem cells, which accompany the global reversion of the somatic epigenome into an ES-like state (13). However, the clinical success with these methods using genetic manipulation must await

* This work was supported by Grants 06122KFDA426 and 07132KFDA688 from Korea Food & Drug Administration in 2006 and 2007. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Fig. S1 and Table S1.

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[3] The abbreviations used are: ES, embryonic stem; EC, embryonic carcinoma; ChIP, chromatin immunoprecipitation; RT, reverse transcription; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; DAPI, 4′,6-diamidino-2-phenylindole; GM, growth medium; RA, all-*trans*-retinoic acid; GFAP, glial fibrillary acidic protein; Q-PCR, quantitative PCR; PI3K, phosphatidylinositol 3-kinase; mTOR, mammalian target of rapamycin; pol, polymerase; Ogn, osteoglycine; Nts, neurotensin; Ngn2, neurogenin2; Gapdh, glyceraldehyde-3-phosphate dehydrogenase.

development of methods that avoid potentially harmful genetic modification.

As an alternative, the epigenome of somatic cells may also be reprogrammed using small molecules that could increase the cellular plasticity of mammalian cells. Such small molecules could offer several advantages that include the ability for temporal, tunable, and modular control of specific proteins involved in dedifferentiation processes without abnormal genetic modification. Recently, Chen *et al.* (14) have screened a library of small heterocyclic compounds, including 2,6-disubstituted purine, for their ability to induce dedifferentiation in somatic mammalian cells, and they identified a purine derivative, reversine, that can increase the plasticity of lineage-committed progenitor cells of mouse myotubes (myoblasts, C2C12 cells). C2C12 myoblasts treated with reversine do not differentiate into myotubes but gain the ability to redifferentiate into other mesodermal lineages, osteoblasts and adipocytes, respectively, when cultured with the appropriate medium (14). Furthermore, reversine has been shown to promote the reprogramming of primary murine and human fibroblasts into myogenic competent cells, which can be then converted into skeletal muscle both *in vitro* and *in vivo* (15). More recently, it has been reported that reversine can increase the plasticity of C2C12 myoblasts at the single-cell level and is active in multiple cell types, including 3T3E1 osteoblasts and human primary skeletal myoblasts. Reversine functions as a dual inhibitor of non-muscle myosin II heavy chain and MEK1, and both activities are required for the function of reversine in dedifferentiation of C2C12 myoblasts. Inhibition of MEK1 and epidermal growth factor and basic fibroblast growth factor results in accumulation of G_2/M phase cells and a decrease in the global acetylation of histone H3 at lysine 9 (16). In addition, inhibition of PI3K blocks the effect of reversine on the redifferentiation potential of C2C12 myoblasts toward other cell types of mesodermal lineages. Although dedifferentiation of C2C12 myoblasts by reversine and the subsequent redifferentiation toward other mesodermal lineages have been shown, the full potential of reversine on dedifferentiation of these cells remains to be addressed.

Toward this end, we have carried out large scale gene expression profiling of reversine-treated C2C12 myoblasts. Multiple specific markers of mesodermal and neural lineages have been identified to be up-regulated in these cells via a specific combination of active histone modifications of these genes. Consistent with the gene expression profiling, the reversine-treated C2C12 myoblasts dedifferentiate into more multipotent progenitor-type cells and can be induced to differentiate into neural and other mesodermal lineage cells. Taken together, these findings provide new insight into the molecular mechanisms by which reversine promotes dedifferentiation of the lineage-committed cells into multipotent progenitor-type cells and suggest a potential rationale for the application of reversine in regenerative medicine.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—The C2C12 cell line, which is mouse myoblasts, was cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen), supplemented with 10% fetal bovine serum (FBS, HyClone Laboratories, Logan, UT) and 1% penicillin/streptomycin (Invitrogen). Transient transfections were performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. C2C12 cells were plated at a seeding density of 5×10^4/10-cm cell culture plates in 15% FBS and antibiotics, and the next day, transfection was performed with Hes6 RNA interference or control RNA (Invitrogen) as indicated in the text. 1 day later, the cells were treated and differentiated as described above according to neural differentiation methods. C17.2 cells were cultured in DMEM with 10% FBS and 5% horse serum (growth medium (GM)) and passaged at the density of 50% confluence every 2 days. To induce differentiation, ~60% confluent cultures were transferred to DMEM containing 2% horse serum (differentiation medium). P19 mouse embryonic carcinoma cells were cultured in α-minimum essential medium supplemented with 10% FBS and 1% penicillin and streptomycin.

Reagents and Antibodies—Reversine was obtained from Calbiochem or prepared by the known procedures (14). LY294002 and rapamycin were obtained from Calbiochem. Commercial primary antibodies were as follows: anti-β-actin, anti-pol II, anti-trimethyl-H3 Lys-4, and H3 Lys-9 (Abcam Inc., Cambridge, MA); anti-MyoD, Akt, phospho-Ser-473 Akt, anti-NFM, anti-NG2, and anti-S100β (Santa Cruz Biotechnology, Santa Cruz, CA); anti-acetyl-histone H4 and H3 (Lys-9/Lys-14) (Upstate Biotechnology, Lake Placid, NY); anti-Tuj1, anti-GFAP, anti-MAP2, and anti-NCAM (Zymed Laboratories Inc.).

RNA Extraction, RT-PCR, and Q-PCR—Total RNA was extracted using easy-Blue reagent (iNtRON Biotechnology, Sungnam, Korea). Integrity of RNA was checked by agarose gel electrophoresis and ethidium bromide staining. Using a RNA PCR kit (Promega, Madison, WI), 1 μg of RNA was used as a template for each RT-mediated PCR. The cDNA was amplified for 25 cycles under the following conditions: melting at 94 °C for 1 min, annealing at 55 °C, and extension at 72 °C for 1 min. Reaction products were analyzed with 1.5% agarose gel electrophoresis. For Q-PCR, template cDNAs were reverse-transcribed from 1 μg of total RNA using oligo(dT) primer and SuperScript II reverse transcriptase (Invitrogen). The PCRs were performed in 20 μl using 1% of the RT reaction, 50 nM of each primer, and SYBR Green master mix (Applied Biosystems). The PCRs were carried out on an ABI PRISM 7000 sequence detection system (Applied Biosystems). Expression levels of Gapdh were used to normalize the expression levels of each sample. Primer sequences used for PCR were as follows: MyoD, 5′-AGTAGAGAAGTGTGCGTGCT-3′ and 5′-ACGACTTCTATGATGATCCG-3′; apoE, 5′-TGCTGTTGGTCACATTGCTG-3′ and 5′-GGAGCTCTGCAGCTCTTCCT-3′; Ogn, 5′-AACCTGTGCAAAGCCAAGTG-3′ and 5′-CCCTTTCCTTGGGCTAAGTG-3′; Ngn2, 5′-GATGCCAAGCTCACGAAGA-3′ and 5′-ACCGTGGAGTTGGAGGATGAC-3′; Hes6, 5′-TTCACCTCTCCCTGCCTTTT-3′ and 5′-TTAATTCGGTTGGAGCATCG-3′; Nts, 5′-GAAAGCCAGGAGAGTCAGGC-3′ and 5′-AGCTGAAGGCAAGAGGAAGC-3′; Irx3, 5′-CCCCTATGGCCAGTACCAGT-3′ and 5′-ATAAGCATTGCCCTCCTCGT-3′; Hes1, 5′-GTCACCTTCCAGTGGCTCCT-3′ and 5′-GTGGGCTAGGGACTTTACGG-3′;

Pax7, 5′-GCCAAGAGGTTTATCCAGCC-3′ and 5′-AGAGGGGTGGACACTTCCAG-3′; GFAP, 5′-TGCTGGCTTCAAGGAGACAC-3′ and 5′-GACATCAGCCAGTTTGGTGG-3′; Tuj1, 5′-TGCTGGCCATCCAGAGTAAG-3′ and 5′-ATGCGTTTGAACAGCTCCTG-3′; Mash1, 5′-GGCTCAACTTCAGCGGCTTC-3′ and 5′-GTTGGTAAAGTCCAGCAGCTC-3′; MAP2, 5′-GCAACGCCAATGGATTTCCA-3′ and 5′-CTCCTGCTCAGGGAATTCCA-3′; NFM, 5′-AGACCTTTGAGGAGAAGCTGG-3′ and 5′-TTCCCTCATATTGCACAAAGG-3′; Ngn1, 5′-GGCTCTGCTGCACTCCCTG-3′ and 5′-TCTCGATCTTCGTGAGCTTG-3′; S100β, 5′-CGCCTGGAGACGCCATCCACG-3′ and 5′-CCTGAAAACTTTGCCCCCTCC-3′; CD44, 5′-GCTCCACCATCGAGAAGAGC-3′ and 5′-ACCAGAAGTTGTGGTCACTCC-3′; and Gapdh, 5′-TGATGACATCAAGAAGGTGAAG-3′ and 5′-TCCTTGGAGGCCATGTAGGCCAT-3′.

Western Blot Analysis—Cell lysates were boiled in Laemmli sample buffer for 3 min, and then 30 μg of each protein were subjected to SDS-PAGE. Proteins were transferred to polyvinylidene difluoride membranes; the membranes were blocked for 30 min in Tris-buffered saline containing 0.1% Tween 20 and 5% (w/v) dry skim milk powder and incubated overnight with primary antibodies. The membranes were then washed with Tris-buffered saline, 0.1% Tween 20, incubated for 1 h with a secondary antibody, and visualized with an enhanced chemiluminescence detection kit (Amersham Biosciences).

Chromatin Immunoprecipitation (ChIP) Assay—Chromatin from 1×10^6 C2C12 cells sheared by a sonicator was precleared with salmon sperm DNA-saturated protein A/G-Sepharose and then precipitated by acetylated histone H3 antibody and others. After immunoprecipitation, recovered chromatin fragments were subjected to PCR (PCR conditions were as follows: 30 cycles with denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 68 °C for 30 s) using primer pairs specific for *MyoD*, *Ogn*, *ApoE*, *Hes1*, and *Hes6* promoter. The primer sequences for each promoter are as follows: MyoD, 5′-CTTTCTTCACCACACCTCTG-3′ and 5′-CGTCTTAACTTTCTGCCACT-3′; Ogn, 5′-TGCCGCAGTTTCAAATATCC-3′ and 5′-GTTTCAGGGCCCAACAGTTT-3′; *ApoE*, 5′-TGAAGGGGGAGAGAACAACC-3′ and 5′-CAAGTCCTTAGCCTCCAGGG-3′; Hes1, 5′-CGGAATCCCCTGTCTACCTC-3′ and 5′-TTTTTCTTCTCAGTCCCCAGAG-3′; and Hes6, 5′-GCCGAGGCTGCTGTCTG-3′ and 5′-TGGGCGTCTGGCCGACA-3′.

cDNA Microarray Analysis—Total RNA was isolated from control and reversine-treated C2C12 cells using easy-Blue reagent (iNtRON Biotechnology) with an additional purification step (RNeasy, Qiagen) before quality assessment (Agilent Bioanalyzer, Palo Alto, CA). Total RNA (2 μg) was reverse-transcribed with the chemiluminescent RT-IVT labeling kit (Applied Biosystems) and hybridized to a 60-mer whole-genome oligonucleotide microarray (Applied Biosystems) containing 33,202 probes representing 29,098 genes. Array hybridization, chemiluminescence detection, image acquisition, and analysis were performed using Applied Biosystems chemiluminescence detection kit and Applied Biosystems 1700 chemiluminescent microarray analyzer according to the manufacturer's protocol. The hybridization reaction was duplicated per sample.

Mesodermal Lineage Differentiation—C2C12 cells were treated with 5 μM reversine for 4 days and washed in DMEM. For adipogenic differentiation, cells were cultured for a further 3 days in DMEM supplemented with 10% FBS, 0.1 μM dexamethasone, 50 μg/ml indomethacin, 0.45 mM 3-isobutyl-1-methylxanthine, 50 μg/ml ascorbate 2-phosphate, and 0.01 mg/ml insulin. Cells were fixed with 4% formalin, washed in 5% isopropyl alcohol and stained for 15 min with Oil-Red-O. For osteogenic differentiation, cells were cultured for 6 days in DMEM supplemented with 10% FBS, 0.1 μM dexamethasone, 50 μM ascorbate 2-phosphate and 10 mM β-glycerophosphatase, replacing the medium every 2–3 days. Extracellular matrix mineralization nodules were visualized by alkaline phosphatase staining.

Induction of Neural Differentiation—C2C12 cells were treated with 5 μM reversine for 4 days or with 20 nM reversine for 2 days and washed in DMEM. For neuronal differentiation, cells were cultured for further 4 days in 1:1 mixture of DMEM/F-12 (Invitrogen) supplemented with N2 and B27 (Invitrogen), replacing the medium every 2–3 days.

Reversine/RA-induced Neural Differentiation—C2C12 or P19 cells were plated at a seeding density of 5×10^4 cells/60-mm poly-D-lysine- (Sigma) and laminin (Invitrogen)-coated dish 1 day before treatment of reversine. After 2 days of incubation of 20 nM reversine, cells were then treated with 0.5 μM all-*trans*-retinoic acid (RA, Sigma) in serum-free medium (DMEM/F-12 media supplemented with the ITS (Sigma) and antibiotics) for 2 days and switched into serum-free medium in the absence of RA with replacement of the medium every 2 days (17). Disruption of the actin cytoskeleton was achieved by adding 2 μg/ml cytochalasin-D (Sigma) or 2% DMSO for 90 min in growth condition and neural differentiation condition.

Immunocytochemistry—For immunocytochemistry, cells were cultured onto coverslips, and after induction of differentiation, cells were fixed with 3% paraformaldehyde, followed by permeabilization with 0.1% Triton X-100, and blocking with bovine serum albumin. The following primary antibodies were used to analyze neuronal and glial differentiation of cells: anti-Tuj1, anti-GFAP, anti-MAP2, anti-NFM, anti-NCAM, anti-NG2, and anti-S100β. Secondary antibodies used were conjugated with Alexa 488 and Alexa 555 (Invitrogen). For the nuclei staining, the cells were incubated in 4 μg/ml of 4′,6-diamidino-2-phenylindole (DAPI) in phosphate-buffered saline for 20 min (Biosciences).

RESULTS

Large Scale Gene Expression Profiling of Reversine-treated C2C12 Cells Identifies Genes Indicative of Multilineage Priming for Mesodermal and Neuroectodermal Lineages—Reversine has been reported to induce dedifferentiation of murine myoblasts into more multipotent progenitor-type cells that can be directed to differentiate not only adipocytes but also osteoblasts (14–16). The processes underlying differentiation and dedifferentiation are precisely controlled by proper gene expression through epigenetic programming and reprogramming of the genome. To evaluate the extent of transcriptional alterations

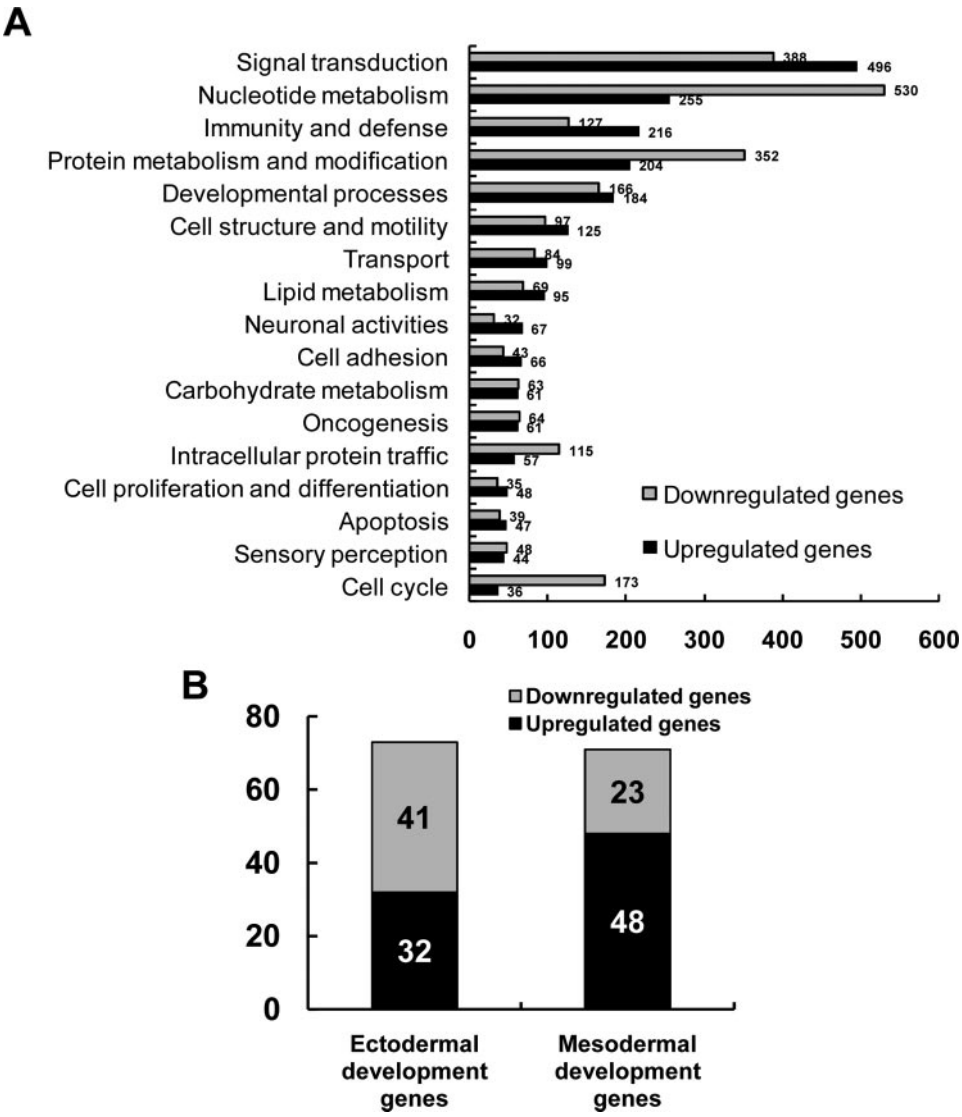

FIGURE 1. **Microarray analysis of gene expression in reversine-treated C2C12 myoblasts.** *A,* C2C12 cells were treated with 5 μM reversine for 4 days, and alteration of gene expression pattern by reversine was determined as described under "Experimental Procedures." Numbers of up- and down-regulated genes more than 2-fold relative to control C2C12 myoblasts by reversine are shown in *black* and *gray*, respectively ($p < 0.05$). *B,* up- and down-regulated genes by reversine were functionally classified into ectodermal and mesodermal development.

elicited by reversine, and to examine the possibility of reversine-treated C2C12 myoblasts to redifferentiate to other lineage cells than mesodermal lineage cells, global gene expression levels were determined by an AB 1700 full genome expression mouse microarray analysis and compared between control and reversine-treated C2C12 cells. Reversine induces up- and down-regulation of 3426 and 3467 genes with more than 2-fold significant changes relative to control C2C12 cells, respectively ($p < 0.05$). Functional classification of the up- and down-regulated genes reveals that most annotated up-regulated genes encode proteins involved in signal transduction, nucleotide metabolism, immunity, protein metabolism, and developmental processes, whereas down-regulated genes are distributed into classes of nucleotide metabolism, signal transduction, protein metabolism, cell cycle, and developmental processes (Fig. 1*A*). Among these, the up- and down-regulated genes related to the developmental process encode elements associated with anterior/posterior patterning, determination of dorsal/ventral axis, ectoderm development, embryogenesis, fertilization, gametogenesis, meiosis, mesoderm development, and segment specification (supplemental Table S1). Notably, expression of genes involved in ectoderm and mesoderm development is significantly changed. In the category of ectoderm-related genes, 32 genes are up-regulated, and 41 genes are down-regulated, and in mesoderm-related genes, 48 genes are increased, and 23 genes are decreased (Fig. 1*B*).

Table 1 lists markers of multilineage differentiation potential for mesodermal and neuroectodermal lineages. The expressions of Ogn and apoE mRNA, which are specific markers of osteogenic and adipogenic lineages, respectively, are markedly up-regulated upon treatment of C2C12 cells with reversine. In addition, genes of procollagen families, specific markers of chondrogenic lineage, are also up-regulated significantly. Interestingly, we also noted the up-regulation of specific markers of neural lineage such as Ngn2, Nts, Irx3 (Iroquois-related homeobox 3), Pax7 (paired box gene 7), Hes1 (hairy and enhancer of split), and Hes6 with more than 2-fold increases. However, we were not able to identify markers of multilineage differentiation potential for endodermal development. Taken together, these results indicate that reversine promotes the up-regulation of markers of multipotency and suggest the establishment of a multilineage priming for neuroectodermal and mesodermal lineages in C2C12 myoblasts treated with reversine.

Reversine Alters Expression of MyoD Gene, and Markers of Mesodermal Lineage Differentiation Potential, ApoE, and Ogn Genes, through Changing Histone Modifications in Their Promoter Regions—To understand the molecular mechanism of the reversine action, we first examined its effect on expression of MyoD, a myogenic specific marker. Treatment of C2C12 myoblasts with 5 μM reversine in growth medium markedly reduces the level of MyoD mRNA and protein in a dose-dependent manner (Fig. 2, *A* and *B*) as reported previously (14). Histone modifications such as acetylation and methylation play an important role in regulation of gene expression through altering chromatin structure (18). To examine whether down-regulation of MyoD expression by reversine is also associated

TABLE 1
Changes in expression level of markers of multilineage differentiation potential by reversine

Name	Accession no.	Description	Fold (up-regulation)
Neurogenic lineage			
Ngn2	NM_009718.2	Neurogenin 2	2.38
Nts	NM_024435.2	Neurotensin	2.40
Hes1	NM_008235.2	Hairy and enhancer of split 1	2.53
Hoxc8	NM_010466.1	Homeobox C8	2.59
Hes6	NM_019479.3	Hairy and enhancer of split 6	2.62
Irx3	NM_008393.1	Iroquois-related homeobox 3	2.86
Hoxa10	NM_008263.1	Homeobox protein A10	3.10
Lhx9	NM_010714.1	LIM homeobox protein 9	3.56
Nr4a2	NM_013613.1	Nuclear receptor subfamily 4, group A, member 2	3.57
Pax7	NM_011039.1	Paired box gene 7	6.07
Atoh8	NM_153778.2	Okadin	14.46
Nr4a1	NM_010444.1	Nuclear receptor subfamily 4, group A, member 1	42.68
Osteogenic lineage			
Ctsk	NM_007802.2	Cathepsin K	2.54
Ogn	NM_008760.2	Osteoglycin	491.50
Adipogenic lineage			
Apobec3	NM_030255.2	Apolipoprotein B editing complex 3	8.35
Apobec2	NM_009694.1	Apolipoprotein B editing complex 2	2.36
Apoa1bp	NM_144897.3	Apolipoprotein A-I-binding protein	3.43
Apobec1	NM_031159.2	Apolipoprotein B editing complex 1	8.62
Apob48r	NM_138310.1	Apolipoprotein B48 receptor	12.14
Apoe	NM_009696.2	Apolipoprotein E	96.46
Chondrogenic lineage			
Col5a2	NM_007737.1	Procollagen, type V, α2	2.55
Col8a1	NM_007739.1	Procollagen, type VIII, α1	2.98
Col16a1	NM_028266.3	Procollagen, type XVI, α1	3.27
Col3a1	NM_009930.1	Procollagen, type III, α1	3.44
Col1a1	NM_007742.2	Procollagen, type I, α1	3.84
Col1a2	NM_007743.1	Procollagen, type I, α2	6.01
Col2a1	NM_031163.1	Procollagen, type II, α1	6.31
Col11a1	NM_007729.1	Procollagen, type XI, α1	11.95
Col12a1	NM_007730.1	Procollagen, type XII, α1	17.42
Col6a2	NM_146007.1	Procollagen, type VI, α2	34.13
Col6a1	NM_009933.1	Procollagen, type VI, α1	44.43

with alteration of histone modifications on the nucleosomes in the promoter region containing muscle-specific CAAT-box (MCAT), and various transcription factor binding sites for MyoD, Sp1, and AP2, we determined the levels of histone H3-Lys-4 and -Lys-9 trimethylation, as well as acetylation of H4 and H3 Lys-9/Lys-14 in the presence or absence of reversine using ChIP analysis. Acetylation of both H3 and H4 is depleted following reversine treatment (Fig. 2*C*). In addition, Lys-4-trimethylated H3, an active marker, on the nucleosomes in the promoter region are enriched in the absence of reversine, whereas reversine treatment results in a significant depletion of Lys-4-trimethylated H3. In contrast, the repressive marker Lys-9-trimethylated H3 is increased in the presence of reversine, which is accompanied by the down-regulation of MyoD expression. Taken together, the results indicate that down-regulation of MyoD expression by reversine might be attributed to depletion of active histone modifications, including acetylated H3, H4, and Lys-4-trimethylated H3, and enrichment of repressive marker Lys-9-trimethylated H3 in the promoter of MyoD.

Expression of markers of multilineage differentiation potential is essential for the establishment of multilineage differentiation priming of multipotent progenitors. Thus, we confirmed the microarray data showing that reversine up-regulates the expression of genes indicative of mesodermal lineage priming, apoE for adipogenic lineage, and Ogn for osteogenic lineage. Treatment of C2C12 myoblasts with reversine causes a significant increase in expression levels of apoE and Ogn as determined by RT-PCR analysis, which are maintained during differentiation (Fig. 2*D*).

Changes in expression level of the genes are accompanied by histone modifications in their promoter regions (Fig. 2*E*). Reversine treatment up-regulates expression of the *ApoE* gene by active histone modifications, including hyperacetylation of H4 and H3Lys-9/Lys-14, enrichment of Lys-4-trimethylated H3, and depletion of repressive marker, Lys-9-trimethylated H3. Similarly, active histone modifications in the promoter of the *Ogn* gene are enriched in the presence of reversine. In parallel, reversine-treated myoblasts can redifferentiate into mesodermal lineage, osteoblast and adipocyte, under specific differentiation conditions (Fig. 2*F*), consistent with the previous report (16). Taken together, these results indicate that increase in plasticity of reversine-treated C2C12 cells for mesodermal lineage might be attributed to up-regulation of genes indicative of mesodermal lineage priming, such as *ApoE* and *Ogn*, through inducing active histone modifications on their promoter regions.

Reversine Up-regulates Specific Markers of Neural Lineage and Induces Active Histone Modifications on Their Promoter Regions—Although reversine has been shown to induce redifferentiation of C2C12 cells into other cell types of mesodermal lineages, including osteoblasts and adipocytes, large scale gene expression profiling of reversine-treated C2C12 cells showing the up-regulation of specific markers of neural lineages leads to an assumption that reversine-treated C2C12 myoblasts might also have a potential to redifferentiate into neural lineage, one of neuroectodermal lineages. To test this, we first confirmed expression of specific markers of neural lineage by RT-PCR, which is known to be expressed before overt neural differenti-

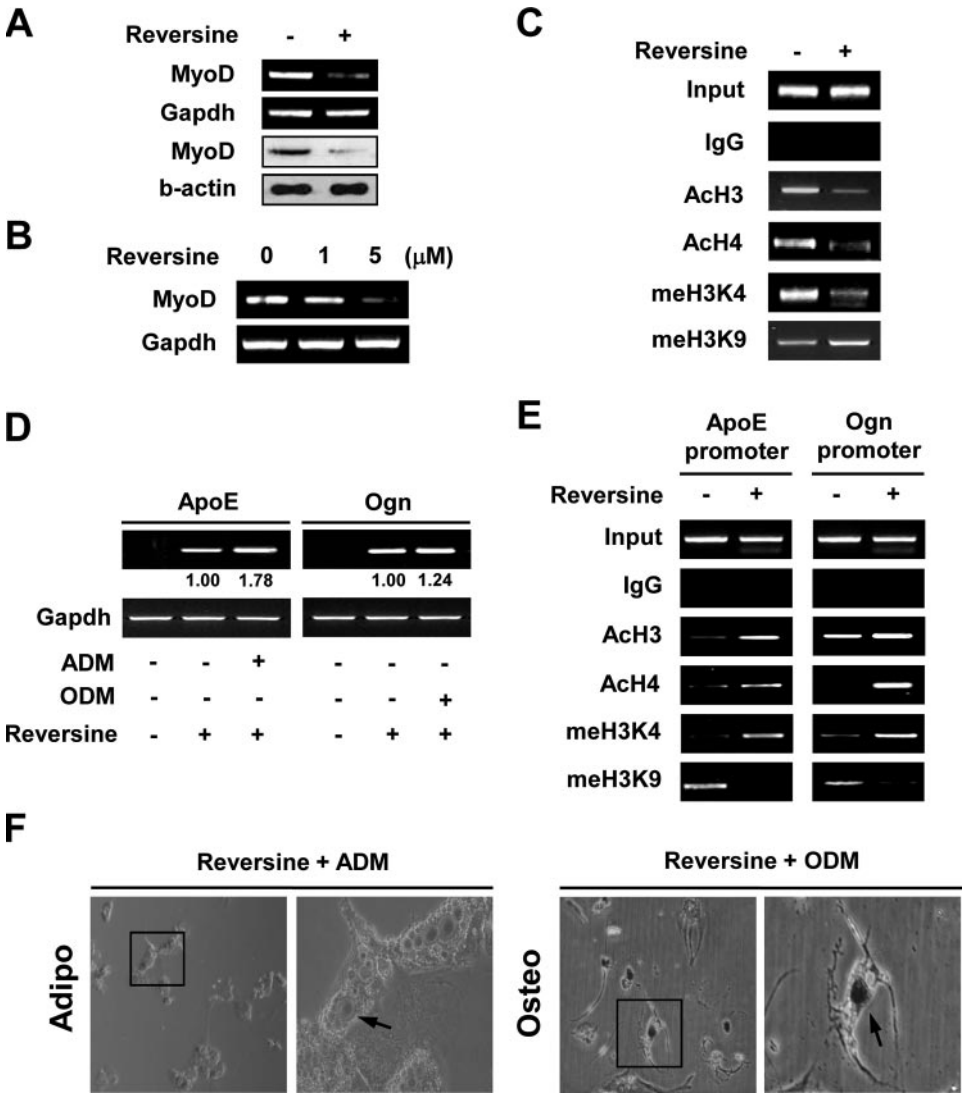

FIGURE 2. **Reversine changes expressional levels of MyoD, Ogn, and apoE through altering histone modifications on their promoters.** *A* and *B,* C2C12 cells were treated with 5 μM or indicated concentrations of reversine for 4 days. The mRNA and protein levels of MyoD were determined as described under "Experimental Procedures." Expression levels of mRNA and protein were normalized to Gapdh and β-actin loading control, respectively. *C,* C2C12 cells were treated with 5 μM reversine for 4 days. Changes of histone modifications in the promoter of *MyoD* gene by reversine was determined by ChIP analysis using anti-acetylated or -methylated histones antibodies as described under "Experimental Procedures." *D,* C2C12 cells were treated with 5 μM reversine for 4 days, and then the mRNA levels of apoE and Ogn were determined as described under "Experimental Procedures." Expression levels of mRNA were normalized to Gapdh loading control. *E,* after treatment of C2C12 cells with 5 μM reversine for 4 days, the histone modifications in the both *ApoE* and *Ogn* gene promoter region were analyzed by ChIP assay with anti-acetylated or -methylated histones antibodies. *F,* C2C12 cells were treated with 5 μM reversine for 4 days and washed and cultured in either adipocyte (*Adipo*) differentiation medium (*ADM*) or osteogenic (*Osteo*) medium (*OM*). Cells were stained with Oil-Red-O or alkaline phosphatase.

ulation of *Hes1* and *Hes6* by reversine is concomitant with histone modifications in their promoter regions (Fig. 3*B*). Reversine treatment induces active histone modifications, including hyperacetylation of H3 and H4, enrichment of Lys-4-trimethylated H3, and depletion of Lys-9-trimethylated H3 in the promoter region of *Hes1* and *Hes6*. Collectively, these results indicate that reversine induces expression of genes indicative of neural lineage priming.

Reversine Enhances a Differentiation Potential toward Neural Lineage Cells—To determine whether in parallel with up-regulation of specific markers of neural lineage, reversine-treated C2C12 myoblasts acquire a differentiation potential for neural lineage, we attempted to induce neural differentiation of reversine-treated C2C12 myoblasts *in vitro*. C2C12 myoblasts were treated with either reversine (20 nM) or DMSO, and then cultured in neural differentiation medium after removing reversine. The induction of a neurogenic and glial phenotype is analyzed by immunostaining with anti-Tuj1, pan-neuronal maker, and anti-GFAP, a glial marker, respectively (Fig. 3*C*). The cells are counterstained with DAPI, and the ratio of GFAP- and Tuj1-positive cells to DAPI-stained cells was quantified. Reversine treatment increases the number of GFAP-positive (56%) and Tuj1-positive cells (35%) (Fig. 3*D*), suggesting differentiation of reversine-treated cells into neural lineages. However, among these, some cells are positive for both anti-GFAP and Tuj1 (23%). Expression of GFAP and Tuj1 is also confirmed by RT-PCR and Western blot (Fig. 3*E*). Similarly, 5 μM reversine also induces redifferentiation of C2C12 myoblasts into neural cell types (supplemental Fig. S1). To further characterize the neural differentiation of these cells, expression of additional neural marker (Mash1), neuronal markers (MAP2, neurofilament middle chain (NFM), Ngn1), and glial markers (S-100β, CD44) are analyzed by using RT-PCR and Q-RT-PCR. As shown in Fig. 3*F*, reversine significantly increases expression of these genes, compared with that of control cells. Upon induction of neural differentiation with RA, a well known inducer of neural differentiation in several cell systems, reversine causes morphological changes and

ation in precursors and performs a determination function in neurogenesis. Consistent with our result obtained from microarray analysis, treatment of C2C12 myoblasts with 5 μM reversine leads to significant up-regulation of *Ngn2*, *Irx3*, *Nts*, *Hes1*, *Hes6*, and *Pax7*. Low concentration (20 nM) of reversine also induces expression of the genes to the level equivalent to that up-regulated by 5 μM reversine (Fig. 3*A*). Similarly, both concentrations of reversine have been reported to have the same effect on the redifferentiation of C2C12 myoblast into osteoblasts and adipocytes (16). Among these, the epigenetic regulation of two genes, *Hes1* and *Hes6*, was examined. Up-reg-

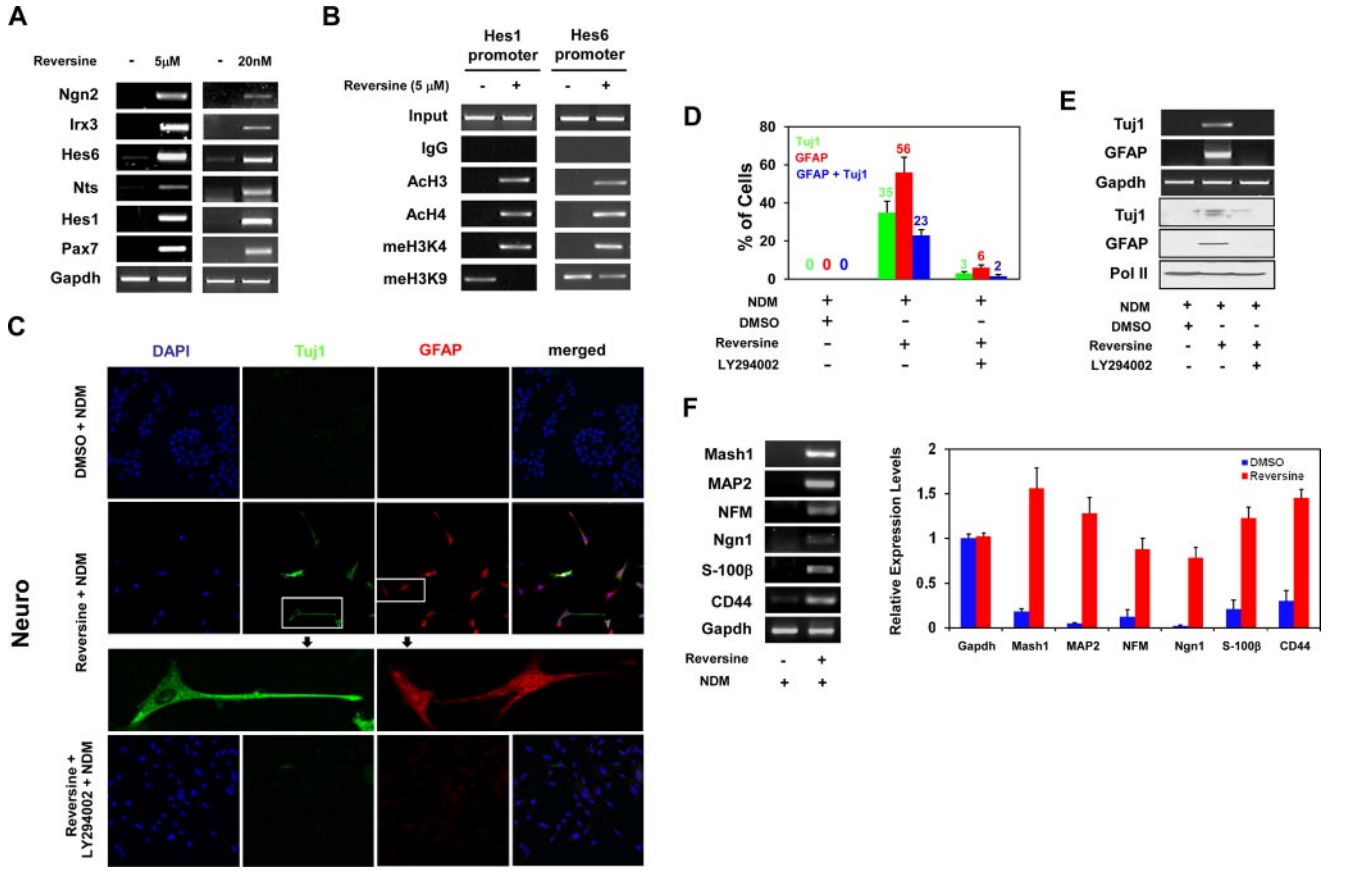

FIGURE 3. **Reversine up-regulates expression of genes indicative of neural lineage priming via inducing active histone modifications, and reversine-treated C2C12 cells redifferentiate into neural lineage cells.** *A,* for RT-PCR, C2C12 cells were treated with 5 μM for 4 days or with 20 nM reversine for 2 days. The mRNA levels of Ngn2, Irx3, Hes6, Nts, Hes1, and Pax7 were determined as described under "Experimental Procedures." Expression levels of mRNA were normalized to Gapdh loading control. *B,* after treatment of C2C12 cells with 5 μM reversine for 4 days, ChIP was performed with anti-acetylated or -methylated histones antibodies. *C,* C2C12 cells were treated with reversine (20 nM) alone or cotreated with LY294002 (50 μM) for 2 days and washed and then further cultured for 4 days in neuronal differentiation medium (*NDM*). Expression of Tuj1 (*green*) and GFAP (*red*) was analyzed by immunocytochemistry as described under "Experimental Procedures." *D,* for quantitative analysis, the cells were counterstained with DAPI (*blue*). Values represent means of triplicate determinations. *E,* mRNA and protein levels of Tuj1 and GFAP were determined as described under "Experimental Procedures." Expression levels of mRNA and protein were normalized to Gapdh and pol II loading control, respectively. *F,* expression of a large panel of neural marker genes was analyzed by RT-PCR and Q-PCR. C2C12 cells were treated with 20 nM reversine for 2 days and then further cultured for 4 days in NDM. Expression level of neural marker gene (*Mash1*), d neuronal marker genes (*MAP2, NFM,* and *Ngn1*), and glial marker genes (*S-100β* and *CD44*) were analyzed as described under "Experimental Procedures." At least three independent cultures were performed.

increases the number of Tuj1- and GFAP-positive cells (Fig. 4*A*). Tuj1-, MAP2-, NFM-, and NCAM-positive cells show an extensive outgrowth of long axon-like processes, similar to that induced by treatment of mouse EC P19 cells with RA or differentiated C17.2 neural precursor cells (19) (Fig. 4, *B* and *C*). GFAP-, NG2-, and S100β-positive cells display bi- or oligopolar-shaped soma with long processes roughly oriented in a radial direction (Fig. 4*B*). The adoption of C2C12 cells to a neural morphology by reversine treatment appears not to be due to a stress response of these cells, because reversine-treated C2C12 cells undergo progressive morphological changes, lasting up to 6 days, and are at least 3-fold larger in size and cell footprint area than control cells as shown with the phase contrast imaging in Fig. 4, *A* and *B*. In contrast, the addition of stressors (2% DMSO or cytochalasin D) to either the growth medium or the neural induction medium causes rapid cellular shrinkage within 90 min, through cytoplasmic retraction toward the nucleus, resulting in a neuron-like phenotype with refractile cell bodies and few fine extended processes (Fig. 4*D*). These results indicate that the neurite outgrowth of the cells by reversine treatment is regulated through a complicated cellular differentiation process rather than a stressor-mediated process and suggest that reversine-treated C2C12 cells regain multipotency toward neurogenic and gliogenic cells.

PI3K Inhibitor Blocks the Effect of Reversine on Redifferentiation into Neural Lineage Cells—Recently, PI3K inhibitor LY294002 has been shown to decrease the reversine-induced differentiation potential toward other cell types of mesodermal lineages (16). Thus, we tested whether the activation of the PI3K signaling pathway is also associated with the reversine-induced increase in cellular plasticity toward neural lineage cells. C2C12 myoblasts were cotreated with 20 nM reversine and 50 μM LY294002 for 2 days, and changes in expression level of specific markers of neural lineage were determined. Reversine treatment leads to a marked increase in the level of phosphorylated Akt, which is antagonized by LY294002 treatment (Fig. 5*A*). Consistent with the result, LY294002 treatment significantly reduces reversine-induced expression of *Irx3*, *Nts*, *Hes1*, and *Hes6* (Fig. 5*B*). In addition, mTOR inhibitor rapamycin also ablates the expression of *Irx3*, *Hes1*, and *Hes6* (Fig. 5*B*),

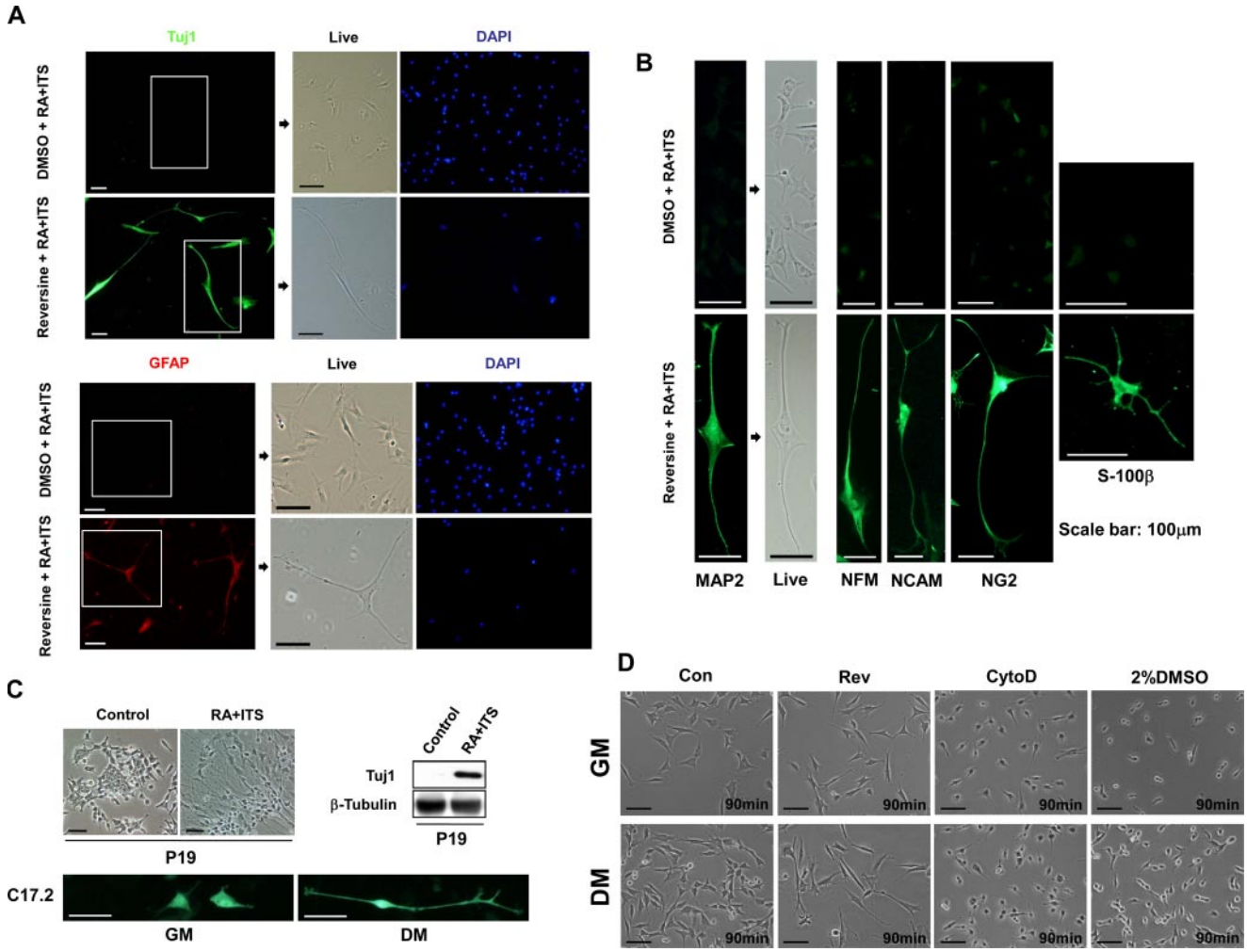

FIGURE 4. **Reversine/RA-induced C2C12 cells express multiple neuronal and glial markers and have neural cell morphology.** *A* and *B*, C2C12 cells were treated with reversine (20 nM) for 2 days and washed and cultured in serum-free DMEM/F-12 containing RA (0.5 μM) and ITS and then further cultured for 4 days in serum-free DMEM/F-12 containing ITS. Expressions of Tuj1, GFAP, MAP2, NFM, NCAM, NG2, and S100β were analyzed by immunocytochemistry as described under "Experimental Procedures." *C*, P19 cells were treated 0.5 μM RA or vector in serum-free DMEM/F-12 containing ITS for 2 days and switched into serum-free DMEM/F-12 in the absence of RA for 4 days. Tuj1 protein expression was performed by Western blot analysis as described under "Experimental Procedures." Green fluorescent protein expression vector was transfected into C17.2 cells and cultured in DMEM with 10% FBS and 5% horse serum (GM). To induce differentiation, ~60% confluent cultures were transferred to DMEM containing 2% horse serum (differentiation medium (*DM*)). *D*, for the live images, C2C12 cells were treated with 20 nM reversine (*Rev*), 2 μg/ml cytochalasin-D (*CytoD*), and 2% DMSO for 90 min in GM (DMEM containing 15% FBS) and differentiation medium (*DM*) (serum-free DMEM/F-12 containing ITS) condition as described under "Experimental Procedures." *Con*, control. *Scale bar*, 100 μm.

indicating the involvement of PI3K-mTOR pathway in the process. LY294002 treatment decreases the level of active histone modifications, including acetylation of H3 and H4, and Lys-4-trimethylated H3 in the promoter region of Hes1 and Hes6, while increasing the level of repressive marker, Lys-9-trimethylated H3 (Fig. 5*C*). Therefore, down-regulation of Hes1 and Hes6 by LY294002 might be caused by alteration of histone modifications in their promoter regions.

In parallel, LY294002 decreases the number of GFAP- (6%) and Tuj1-positive cells (3%) (Fig. 3, *C* and *D*). Expression of GFAP and Tuj1 was further confirmed by RT-PCR and Western blot (Fig. 3*E*). Consistent with the results, Hes6 knockdown with siRNA suppresses the expression of Tuj1 and GFAP (Fig. 5*D*), indicating that increase in the plasticity toward neural lineage by reversine is mediated in part by PI3K pathway-dependent expression of Hes6. Taken together, the results suggest that the activation of PI3K signaling pathway is required for the reversine-induced differentiation potential toward neural lineage cells.

DISCUSSION

Dedifferentiation of lineage-committed cells into stem cells has attracted much attention, because cells dedifferentiated through epigenetic reprogramming will not be rejected by the donor, and thus have the potential for customized transplantation therapy. However, the therapeutic application of dedifferentiation using approaches such as somatic cell nuclear transfer and fusion of lineage-committed cells with ES cells has been hindered by technical complication as well as ethical objections. Recently, as an alternative, a synthetic small molecule reversine has been demonstrated to induce dedifferentiation of terminally differentiated murine myoblasts, and primary murine and human dermal fibroblasts, which can increase their plasticity toward mesodermal lineage cells (15). Here we show

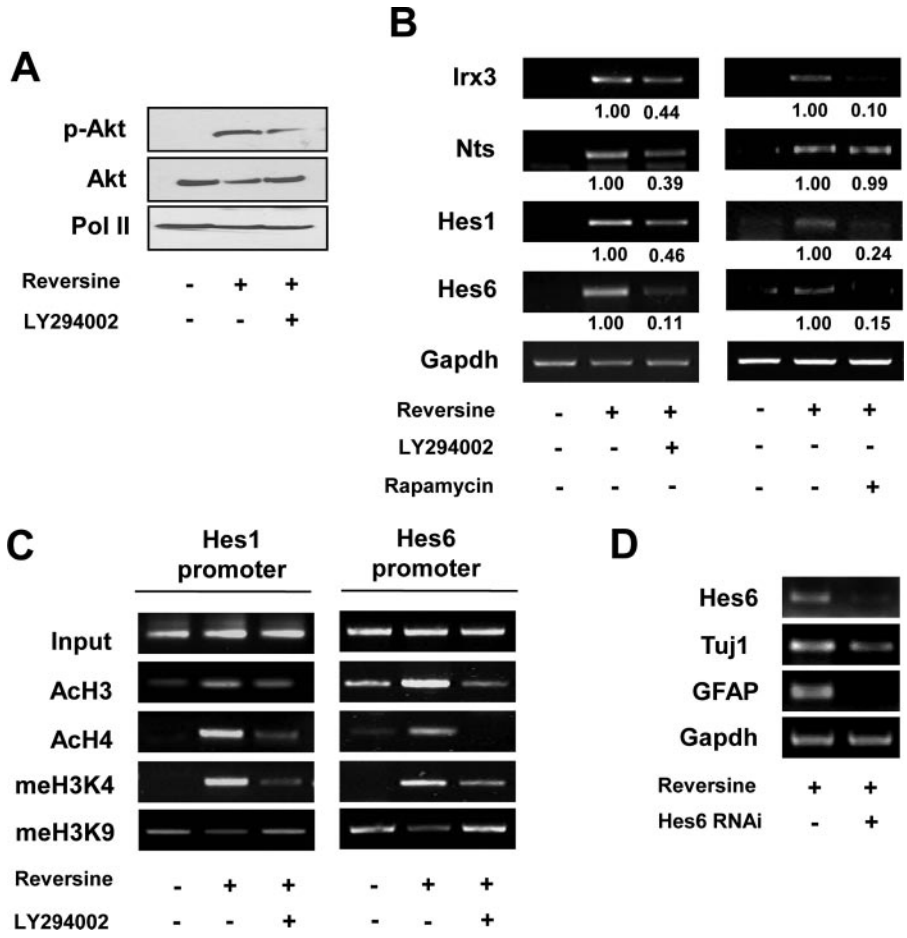

FIGURE 5. **PI3K signaling is required for reversine-induced increase of multipotency toward neural lineage cells.** C2C12 cells were treated with reversine (20 nM) or cotreated with either LY294002 (50 μM) or rapamycin (100 nM) for 2 days. *A,* protein levels of Akt and phosphorylated Akt were determined as described under "Experimental Procedures." Expression levels of protein were normalized to pol II loading control. *B,* mRNA levels of Irx3, Nts, Hes1, and Hes6 were determined as described under "Experimental Procedures." Signals of the lanes were quantified by densitometry. *C,* changes of histone modifications in the promoter region of Hes1 and Hes6 by reversine were determined by ChIP analysis using anti-acetylated or -methylated histones antibodies as described under "Experimental Procedures." *D,* Hes6 RNA interference (*RNAi*) or control RNA was transfected into C2C12 cells. 1 day later, cells were treated with reversine (20 nM) for 2 days and then further cultured for 4 days in NDM. The mRNA levels of Hes6, Tuj1, and GFAP were determined as described under "Experimental Procedures."

that reversine increases the plasticity of lineage-committed murine myoblasts toward neuroectodermal lineages, which is associated with changes in the expression pattern of genes involved in specification of neuroectodermal lineages through inducing a combination of histone modifications in the promoter regions of these genes. In parallel, the reversine-treated C2C12 cells redifferentiate into neurons and glia, under appropriate stimuli, and this process is associated with the PI3K signaling pathway. These findings suggest that the establishment of a multilineage priming for neuroectodermal lineage by up-regulation of priming genes for neuroectodermal lineages might be responsible for dedifferentiation and an increase in plasticity of the cells.

Neuroregeneration has been intensively studied for the potential therapeutic use to repair and restore damaged or diseased neurons. Manipulation of various factors such as epidermal growth factor and basic fibroblast growth factor that control the ability of neural stem cells in the adult brain leads to regeneration of neurons (20). In addition, C2C12 myoblasts can be also converted to the neuronal phenotype by expression of REST target genes via stable expression of a plasmid, REST-VP16, generated by replacing the repressor domains of REST/NRSF with the activation domain of the herpes simplex virus protein VP16 (21). Although C2C12 myoblasts dedifferentiated by reversine have been shown to be able to redifferentiate only to mesodermal lineage cells until now, we provide the first evidence that reversine increases the plasticity of lineage-committed cells C2C12 myoblast toward neuroectodermal lineage. Global gene expression analysis of reversine-treated C2C12 myoblasts using cDNA microarray technology reveals that reversine also induces up-regulation of markers of neural lineages such as Hes1, Hes6, Ngn2, Atoh8, Pax7, Irx3, Hoxa10, Hoxc8, Evx2, Zfh4, Lhx9, Cntf, and Erg2. Among these, neural basic helix-loop-helix transcription factors, Ngn2, Hes1, Hes6, and Atoh8, play essential roles in neurogenesis. Ngn2 and Atoh8 regulate many aspects of neurogenesis, including neural fate specification, neuronal differentiation, and migration of neurons (22–25). Neural basic helix-loop-helix factors can promote neuronal differentiation of non-neural cells when expressed ectopically in frog, fish embryos, and uncommitted P19 mouse EC cells (26–29). Hes1 is highly expressed in neural stem cells and functions as a main effector of the Notch signaling pathway in neural stem cell maintenance (30–31). Notch/Hes signaling may function to maintain a balance between the number of developing neurons and progenitors during cortical development (31–32). Moreover, Notch/Hes signaling promotes glial and astrocyte cell fate, while inhibiting neuronal differentiation. Hes6 is expressed both in undifferentiated neural progenitors as well as differentiated neurons, and its expression is induced by the proneural basic helix-loop-helix proteins but not by Notch signaling. Hes6 acts as a positive regulator of neuronal differentiation by antagonizing the Hes1 function via heterodimerization with Hes1 (33). Induction of these genes by reversine suggests acquisition of the neurogenic and gliogenic identity. Indeed, consistent with up-regulation of neuronal and gliogenic lineage-specific genes, reversine-treated C2C12 myoblasts gained a differentiation potential for neurogenic and gliogenic lineages, as seen by the expression of neuronal markers (Tuj1, MAP2, NFM, and Ngn1) and glial markers (GFAP, S-100β, and CD44), respectively (Fig.

3 and 4). In parallel, Tuj1-, MAP2-, NFM-, and NCAM-positive cells show long axon-like processes, similar to that induced by treatment of mouse EC P19 cells with RA or differentiated C17.2 neural precursor cells (19), but quite different from that induced by disruption of the cytoskeleton in response to stressors (2% DMSO, cytochalasin D) (Fig. 4*D*). Exposure of C2C12 cells to stressors (2% DMSO or cytochalasin D) in the presence or absence of serum induces rapid cellular shrinkage within 90 min, with retraction of the cell edge toward the nucleus, leaving only few fine neurite-like processes, which leads to reduction of cellular footprint area as reported previously (34–35). In contrast, reversine-treated C2C12 cells undergo progressive morphological changes, lasting up to 6 days, after replacing the medium with the neural induction medium, and are at least 3-fold larger in size and cell footprint area than control cells as shown with the phase contrast imaging in Fig. 4, *A* and *B*, representing the neurite outgrowth and regulated steps in a complicated cellular differentiation process. Furthermore, the removal of reversine from the culture medium after treatment of the cells for 2 days does not affect the morphology of the cells, whereas the neuron-like morphology induced by stressor treatment returns to the spread shape within 30 min, when stressors are removed from the medium (data not shown). The notion is further supported by specific expression of MAP2 in reversine-treated C2C12 cells cultured in neural differentiation medium (Fig. 4*B*), because the MAP2-dependent process formation is an active extension process, unlike the formation of neurite-like structures in various cells in response to stressors that develop through retraction of the cytoplasm (35). In addition, the observation showing that the expression of neuronal marker Tuj1 and glial marker GFAP is dependent of PI3K-mTOR signaling pathway and Hes6 expression (Fig. 3*E* and Fig. 5*D*) indicates an *in vitro* analogue of the systematic gene expression, which is seen during differentiation but not the aberrant gene expression in response to stressors. Similarly, GFAP-, NG2-, and S100β-positive cells display bi- or oligopolar-shaped soma with long processes roughly oriented in a radial direction and increased cellular footprint area, different from that observed in cells exposed to stressors. A recent study demonstrating that a population of brain parenchymal cells coexpressing glial and neuronal markers is able to differentiate into cells of neuronal and glial lineages indicates that some cells coexpressing both Tuj1 and GFAP in reversine-treated C2C12 cells might represent a not fully differentiated neural progenitor under the neural differentiation condition (36). Therefore, the protocol for the neural differentiation employed in this study has to be further optimized to induce the fully differentiated neural cells with the full functionality. In this study, we cannot note the up-regulation of genes characteristic of neither undifferentiated ES cells nor endodermal lineage cells. Thus, reversine seems to reprogram the transcriptional signature similarly to somatic stem cells such as mesenchymal and neural stem cells. Collectively, these results demonstrate that reversine can induce dedifferentiation of C2C12 myoblasts to more multipotent progenitor-type cells, representing one of the major events that occur during dedifferentiation.

An indicator of dedifferentiation is the down-regulation of genes indicative of a differentiated state. Among differentially expressed genes by reversine, MyoD has been shown to act as a master switch for differentiation of many different lineages and differentiation states to skeletal muscle through initiating a complex program of differentiation (37–41). Reversine treatment leads to a decrease in the level of MyoD mRNA and protein in a dose-dependent manner (Fig. 2, *A* and *B*), as reported previously. The other indicator of dedifferentiation is the up-regulation of markers of multilineage differentiation potential. Indeed, reversine is able to up-regulate markers of osteogenic (Ogn, Ctsk, and Tnfsf11), adipogenic (apoA2, apoE, and apoC1), and chondrogenic (Col4A3, Col5A2, Col8A1, Col11A1, Agc1, Dspg3, and Fn1) lineages. Consistent with our results, these markers have been shown to be up-regulated in epithelial 293T cells treated with an extract of undifferentiated human NCCIT carcinoma cells, which leads to dedifferentiation of a differentiated cell toward pluripotency (42). Thus, up-regulation of these genes by reversine suggests the establishment of a multilineage priming. Indeed, reversine-treated C2C12 myoblasts can redifferentiate into mesodermal lineage cell types, adipocyte and osteoblast, with high efficiency as reported previously (14, 16). We also expect that the cells might have the differentiation potency toward other mesodermal lineage, chondrogenic lineage, because of up-regulation of markers of chondrogenic lineage.

Histone modifications most likely consist of both acetylation and methylation, which regulate chromatin structure and function (43–44). Generally, acetylation of H3 and H4 in the regulatory regions of genes is associated with gene expression by inducing active chromatin structure. In contrast, a variety of histone methylation at various lysines constitutes the more complex histone code. Recent progress has highlighted the importance of three histone lysine methylations in epigenetics, whereas methylation of histone 3 lysine 9 (H3-Lys-9) and H3-Lys-27 directs gene silencing, methylation of H3-Lys-4 is associated with gene expression. Changes of expression pattern of the genes *MyoD*, *ApoE*, *Ogn*, *Hes1*, and *Hes6* in reversine-treated C2C12 myoblasts are accompanied by alteration of histone modifications on the promoter region. Reversine treatment leads to depletion of active marker (hyperacetylation of H3 and H4, Lys-4 trimethylation of H3) and enrichment of repressive marker (Lys-9 trimethylation of H3) in the promoter region of *MyoD*, indicating that reprogramming of the *MyoD* gene with repressive marker in the promoter region of MyoD might be responsible for down-regulation of *MyoD* expression by reversine (Fig. 2*C*). In addition, histone modifications appear to play an important role in up-regulation of *Hes1*, *Hes6*, *ApoE*, and *Ogn.* Reversine has ability to induce the formation of active markers on the promoter regions of *Hes1* and *Hes6* genes, such as hyperacetylation of H3 and H4, and Lys-4 trimethylation of H3, while suppressing the formation of repressive marker, Lys-9 trimethylation of H3 (Fig. 3*B*). Similarly, histone H3 and H4 on the promoter of apoE and Ogn are modified by reversine (Fig. 2*E*). Although another repressive marker, Lys-27 trimethylation of H3, has been shown to play a role in regulation of various genes involved in development, the modification in the promoter region of these genes is not detected under our conditions. Recent study has shown that reversine decreases the level of global acetylation of H3-Lys-9/Lys-14 in C2C12 myo-

blasts (16). However, reversine treatment leads to the local hyperacetylation of histone H3 and H4 on the promoter region of the up-regulated genes as confirmed by ChIP analysis. Thus, the results indicate that up-regulation of these genes by reversine might be due to reprogramming of each gene via an enrichment of active histone modifications, including acetylated H4 and Lys-4-trimethylated H3 and depletion of repressive marker, Lys-9 trimethylation. Furthermore, inhibition of PI3K antagonizes reversine-induced up-regulation of priming genes, *Hes1* and *Hes6*, for neuroectodermal lineages, which is accompanied by inducing repressive histone modifications in the promoter region. Thus, the elucidation of the relationship between epigenetic modifications and modulation of signaling pathway induced by reversine would be of great interest.

Collectively, these findings support the hypothesis that reversine increases multipotency toward neuroectodermal lineage through up-regulation of genes indicative of neural lineage priming via inducing a specific combination of active histone modifications. The findings provide new insight into molecular mechanisms by which reversine promotes dedifferentiation of lineage-committed cells to more multipotent progenitor-type cells and suggest a mechanistic rationale for the application of reversine in regenerative medicine.

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