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ORIGINAL PAPER

# Heat Shock Protein 90 is Required for cAMP-Induced Differentiation in Rat Primary Schwann Cells

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## Abstract

Schwann cells (SCs) play an important role in producing myelin for rapid neurotransmission in the peripheral nervous system. Activation of the differentiation and myelination processes in SCs requires the expression of a series of transcriptional factors including Sox10, Oct6/Pou3f1, and Egr2/Krox20. However, functional interactions among several transcription factors are poorly defined and the important components of the regulatory network are still unknown. Until now, available evidence suggests that SCs require cAMP signaling to initiate the myelination program. Heat shock protein 90 (Hsp90) is known as a chaperone required to stabilize ErbB2 receptor. In recent years, it was reported that cAMP transactivated the ErbB2/ErbB3 signaling in SCs. However, the relationship between Hsp90 and cAMP-induced differentiation in SCs is undefined. Here we investigated the role of Hsp90 during cAMP-induced differentiation of SCs using Hsp90 inhibitor, geldanamycin and Hsp90 siRNA transfection. Our results showed that dibutyryl-cAMP (db-cAMP) treatment upregulated Hsp90 expression and led to nuclear translocation of Gab1/ERK, the downstream signaling pathway of the ErbB2 signaling mechanism in myelination. The expression of myelin-related genes and nuclear translocation of Gab1/ERK following db-cAMP treatment was inhibited by geldanamycin pretreatment and Hsp90 knockdown. These findings suggest that Hsp90 might play a role in cAMP-induced differentiation via stabilization of ErbB2 and nuclear translocation of Gab1/ERK in SCs.

**Keywords** cAMP · SC differentiation · ErbB2 · Gab1 · Hsp90

## Introduction

Schwann cells (SCs) are the glial cells of the peripheral nervous system (PNS), which form myelin-wrapping large-caliber axons [1]. The myelination of PNS starts around birth and is regulated by a sequential cascade of the transcription factors (TFs) including Pou3f1 (POU domain class 3 TF; Oct6 (octamer-binding TF 6); Pou3f2 (Brn2); Egr2 (early growth response protein 2; Krox20); and Sox10 (SRY-related HMG-box-10) [2]. Axonal signals from neuregulin1 (NRG1) and important signals from the extracellular matrix mediated by integrin and G-protein-coupled receptors (GPR126) regulate the entry into terminal Schwann cell differentiation program [3, 4]. It has been shown that cyclic adenosine monophosphate (cAMP) analogues induce Krox20 and other differentiation markers in rat SCs [5, 6]. Protein kinase A (PKA) is the main downstream molecule of cAMP and plays a crucial role in myelination of SCs [7]. NRG1 type III regulates SC proliferation, migration and myelination via binding to receptor tyrosine kinase ErbB2/3 receptors [8]. A previous study reported that NRG1 type III is required for Oct6 activation and myelination [9]. Extracellular signal regulated kinase (ERK)-dependent pathways are involved in the NRG1-induced Krox20 expression, which is an essential TF for the myelination of peripheral nerves [10, 11]. Recent reports suggest that PKA directly transactivated the ErbB2-Grb2-associated binder 1 (Gab1) axis independent of

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NRG1 type III [12]. Gab1 is a scaffolding protein involved in the signal transduction of NRG1-induced peripheral nerve myelination [13]. However, the detailed molecular interactions between cAMP and ErbB2-Gab1 signaling in SC differentiation and myelination were not completely defined.

It is known that heat shock protein 90 (Hsp90) is involved in neuronal migration [14] and acts as a chaperone in the folding or conformational regulation of molecules in central signal transduction [15]. The two isoforms of human Hsp90 including Hsp90α (gene Hsp90AA1) and Hsp90β (gene Hsp90AB1) are expressed from separate genes, and both isoforms are referred to as Hsp90 [16]. Their functions appear to be redundant; however, a few differences exist. Hsp90β is constitutively expressed in all cells, whereas Hsp90α is induced during stress [16]. It was reported that ErbB2 and ErbB3 interact directly with Hsp90 [17–19]. The ErbB2 function was also regulated by Hsp90 [17, 20]. Several studies have demonstrated the implications of Hsp90 in peripheral nerve myelination. For example, a previous study demonstrated that small molecule inhibitors of Hsp90 such as EC137 enhanced myelination via upregulation of Hsp70 in a PMP22-overexpressing neuropathic model [21]. Urban et al. demonstrated that KU-32, a small molecule inhibitor of Hsp90, decreased NRG1-induced demyelination in an Hsp70-dependent manner [22]. In addition, Li et al. showed that the induction of Hsp70 by KU-32 is required to prevent NRG1-induced demyelination by blocking c-jun expression and phosphorylation [23]. These data suggest that Hsp90 inhibitor may antagonize the expression of the negative regulators of myelination and facilitate the treatment of demyelinating neuropathies.

In the present study, we have investigated the role of Hsp90 during the cAMP-induced differentiation in rat primary SCs using a chemical inhibitor of Hsp90, geldanamycin (GA), and Hsp90 siRNA transfection. We found that cAMP transactivation of ErbB2 requires Hsp90 and the Hsp90-dependent transactivation regulates myelination via Gab1/ERK nuclear translocation in cultured primary SCs.

## Materials and Methods

### Reagents

$N^6$, 2′-*O*-dibutyryladenosine 3′,5′-cyclic monophosphate sodium salt (db-cAMP; cell-permeable analogue of cAMP, Sigma-Aldrich), $N^6$-benzoyladenosine-3′,5′-cyclic monophosphate (6-Bnz-cAMP) and 8-(4-chlorophenylthio)-2′-*O*-methyladenosine-3′,5′-cyclic monophosphate (8-CPT-2Me-cAMP) were purchased from Biolog Life Science Institute, Bremen, Germany. H-89 and PD98059 were ordered from Merck Millipore, USA. PKI166 hydrochloride and Rp-cAMPS (triethylammonium salt) were obtained from R&D Systems, USA. U0126 monoethanolate was procured from Sigma-Aldrich, USA. Hoechst 33342 was obtained from Sigma-Aldrich, USA. Antibodies against Oct6 (SC-11661), MPZ (SC-18533), Hsp90 (SC-13119), and p-Neu (SC-12352) were ordered from Santa Cruz Biotechnology, USA. The monoclonal anti-MAG antibody (SAB1402258) was obtained from Sigma-Aldrich, USA. Antibodies against c-Jun (#9165), Gab1 (#3232), p-Gab1 (#3231), ERK1/2 (#4696), p-ERK1/2 (#9101), PKA C-α (#4782), and phospho-PKA substrate (p-PKA substrate; #9624) were procured from Cell Signaling Technology, USA. Anti-Krox20 antibody (#PRB-236P) was ordered from Covance, NJ, USA. Anti-ErbB2 antibody (MA5-13675) was obtained from Thermo Fisher Scientific, Chicago, IL, USA. Anti-S100β antibody (ab52642) was obtained from Abcam, UK. Alexa Fluor 488-conjugated donkey anti-mouse (A21202) or anti-rabbit IgG (A21206) were supplied by Invitrogen, USA. Cy3-conjugated anti-mouse (AP192C) or anti-rabbit IgG (AP182C) was provided by Merck Millipore, USA. Unless stated otherwise, all other chemicals were purchased from Sigma-Aldrich.

### Primary Cultures of SCs

Primary rat SCs were isolated from neonatal rats based on our previous report [13]. In brief, sciatic nerves were isolated from Sprague-Dawley (SD) neonatal rats (on postnatal days 4–5, provided by Orient). The collected nerves were treated with collagenase (Sigma) at 37 °C for 15 min and digested with 0.05% Trypsin-EDTA (Gibco) at 37 °C for 10 min. Single cells were obtained by gentle pipetting and maintained in DMEM containing 10% fetal bovine serum (FBS) in poly-D-lysine hydrobromide (PDL, Sigma-Aldrich)-coated petri dish. Subsequently, the culture medium was replaced by SC medium (DMEM containing 1% FBS, N-2 supplement (Gibco), 5 μM forskolin (Calbiochem, Gibbstown, NJ) and 30 ng/mL NRG1 (human NRG1-β1 extracellular domain, R&D Minneapolis, MN) to promote cell proliferation. All experiments in this study were performed using SCs at passages 2 to 4. More than 95% of SC purity was confirmed based on their morphology and S100 immunoreactivity.

### Pharmacological Treatments of the Cultured SCs

The differentiation of cultured SCs was induced with db-cAMP after pretreating the cells with DMEM containing 1% FBS for 24 h to obtain the immature phenotype. Next, 500 μM db-cAMP was added to the cultures for 48 h to induce differentiation. To determine the effects of PKA inhibitors (H-89, Rp-cAMPS), ErbB2 inhibitor (PKI166), Hsp90 inhibitor (Geldanamycin; GA), ERK inhibitor (PD98059), and MEK1 inhibitor (U0126) on SC differentiation, the cells were pretreated with H-89 (5 μM), Rp-cAMPS (50 μM),

PKI166 (5 μM), GA (50 nM), PD98059 (100 μM), or U0126 (10 μM) for 1 h before db-cAMP treatment. The concentrations of db-cAMP, 6-Bnz-cAMP, and 8-CPT-2Me-cAMP used in this study were determined by the expression of differentiation markers (Oct6, Krox20, MPZ, and MAG) and cytotoxicity at several concentrations based on the concentration of the published reports [12]. The concentration of each inhibitor (H-89, Rp-cAMPS, PKI166, PD98059, or U0126) used in this study was determined according to the phosphorylation of each target proteins (PKA substrate, ErbB2, and ERK) at several concentrations based on published reports [12]. The concentration of GA used in this study was determined by the expression of ErbB2 and cytotoxicity at several concentrations (25, 50, and 100 nM).

### Total Protein Extraction

Cells were lysed as previously described [24]. Cells were lysed by boiling in 2× sodium dodecyl sulfate (SDS) lysis buffer. The lysates were centrifuged at 13,000 rpm for 10 min at 4 °C. Supernatants were collected and the protein concentration was quantified using a Protein Assay Kit (Bio-Rad). The lysates were cooled to room temperature followed by Western blot analysis.

### Western Blot Analysis

Western blot analysis was performed as previously described [25]. The electrophoresis of 25 μg protein samples was conducted using 8, 10 or 12% SDS-PAGE. Proteins were transferred to nitrocellulose membranes (Amersham Life Science, Inc., Piscataway, NJ, USA) after electrophoresis. Blots were blocked overnight in 5%skim milk in PBS at 4 °C and subsequently probed with the primary antibody for 1 h. After washing, the blots were probed with a secondary antibody for 2 h. After washing, the proteins were detected with ECL detection reagents (Amersham, Buckinghamshire, UK) following the manufacturer's instructions. The blots were also probed with a monoclonal anti-β-actin antibody (Sigma, St. Louis, MO, USA).

### PKA Catalytic Subunit α (PKA Cα) siRNA Transfection

The PKA Cα knockdown was performed using the ON-TARGETplus Human PRKACA siRNA SMART pool (catalog number L-004649000005) purchased from Dharmacon-Horizon Discovery (USA). Rat primary SCs ($2\times10^5$ cells/well) were seeded and cultured overnight in 12-well plates. The siRNAs were transfected with Lipofectamine 3000 transfection reagent (Invitrogen, USA) into SCs using PRKACA siRNA SMART pool reagents according to the manufacturer's instructions. Cells transfected with the ON-TARGETplus non-targeting control (NC) siRNA (catalog number D-0018101020) were used as the control. After transfection, the cells were cultured for 6 h in the growth medium, followed by treatment with 500 μM db-cAMP for 48 h. Finally, the cells were subjected to western blot analysis.

### Hsp90 siRNA Transfection

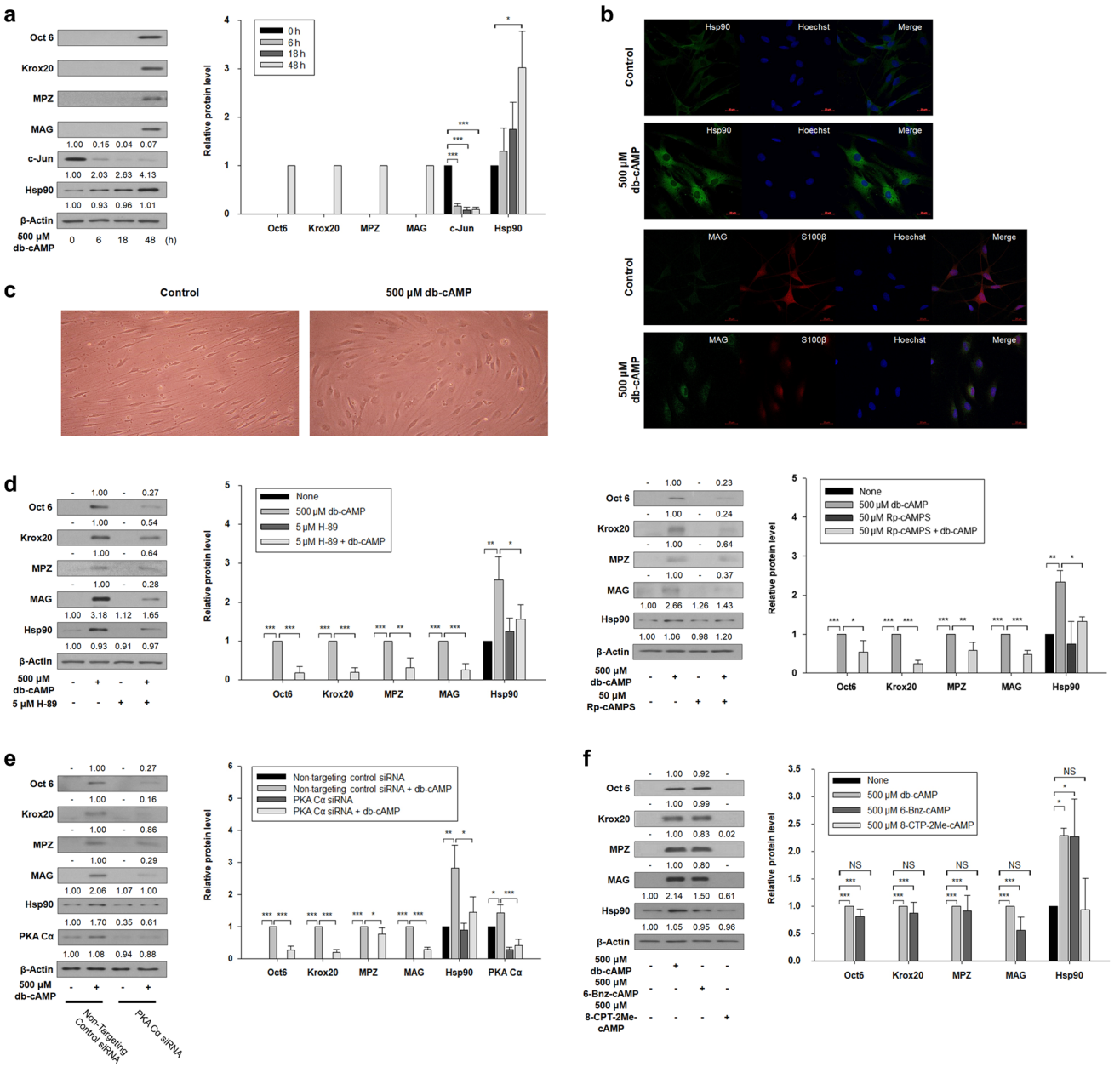
To perform the Hsp90 knockdown, Accell small interference RNAs (siRNAn) against Hsp90aa1 (catalog number E-094085-00-0010) and Hsp90ab1 (catalog number E-102259-00-0010) were purchased from Dharmacon-Horizon Discovery (USA). Rat primary SCs ($2\times10^5$ cells/well) were seeded and cultured overnight in 12-well plates. The siRNAs were transfected with the Lipofectamine 3000 transfection reagent (Invitrogen, USA) into SCs using Accell siRNA reagents according to the manufacturer's instructions. Cells transfected with the NC siRNA (catalog number D-001910-10-20) were used as the control. After transfection, the cells were cultured for 6 h in the growth medium, followed by treatment with 500 μM db-cAMP for 48 h. Finally, the cells were analyzed via immunofluorescence staining and western blot analysis.

### Gab 1 shRNA Transfection

When primary SC reached 70% confluency, lentiviral transduction particles expressing Gab1-shRNA (Gab1 MISSION shRNA Lentiviral Transduction Particles; Sigma-Aldrich, USA) or scrambled shRNA (MISSION® pLKO.1-puro Empty Vector Control Transduction Particles; Sigma-Aldrich, USA) were added for 18–24 h. The medium was replaced with a fresh DMEM containing 10% FBS and cultured for another 24 h. The medium was replaced with the growth medium (DMEM containing 1% FBS, 30 ng/mL NRG1, 5 μM forskolin) for 24 h and db-cAMP for 48 h. The cells were analyzed via immunofluorescence staining and western blot.

### Immunoprecipitation and Immunoblotting

Rat primary SCs (control and db-cAMP-treated) were lysed in 500 μl of RIPA lysis buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7.2, 0.1% SDS, 1% Triton X-100, 5 mM EDTA, 1× protease inhibitor, 1 mM PMSF, 100 μM $Na_3VO_4$, and phosphatase inhibitor cocktail). The supernatants were collected by centrifugation at 13,000 rpm for 10 min at 4 °C, and the protein concentration was determined by Bradford assay (Bio-Rad, Hercules, CA). For immunoprecipitation, 0.5–0.6 mg of protein diluted in lysis buffer was incubated with a Hsp90 antibody (Santa-Cruz Biotechnology, USA), or Gab1 antibody (Cell Signaling Technology, USA) at 4 °C overnight. The immune complexes were recovered with




Protein G Plus-agarose (Santa Cruz Biotechnology, USA) for 2 h with rotation. Immunoprecipitates were subjected to SDS-PAGE and western blot analysis using an antibody to Hsp90, Gab1, or ErbB2.

## Immunofluorescence Staining

Rat primary SCs were fixed and permeabilized with 1% formaldehyde/0.3% Tween 20 in PBS for 10 min at room temperature. Subsequently, the cells were washed three times with PBS and incubated with 0.2% Triton/PBS for 10 min, followed by PBS washing and 1 h blocking in 10% BSA/PBST. After blocking, the cells were incubated overnight with a primary antibody at 4 °C. Cells were visualized using Alexa Fluor 488- or Cy3-conjugated donkey anti-mouse or anti-rabbit IgG (Invitrogen, USA, Merck Millipore, USA, respectively). Nuclei were counterstained with Hoechst 33342 (Sigma-Aldrich). The samples were mounted with glycerol, and analyzed with a confocal microscope (Carl Zeiss LSM800; Carl Zeiss, Thornwood, NY, USA) equipped with a 40× C-Apochromat objective. As a negative control, cells were treated similarly but with the primary antibodies omitted.

◂**Fig. 1** Dibutyryl-cAMP (db-cAMP) treatment upregulates hsp90 expression by PKA and induces differentiation in rat primary Schwann cells (SCs). **a** Left panel: Rat primary SCs were treated with 500 μM db-cAMP for indicated times. Protein lysates were prepared and used for western blot analysis with the corresponding antibodies. β-actin was used as a loading control. The blot is representative of three separate experiments. Densitometry results are expressed above the bands. Right panel: Relative protein level compared with β-actin by densitometry was expressed as average ± SD (n = 3). *$P < 0.05$, ***$P < 0.001$. **b** Rat primary SCs were treated with or without 500 μM db-cAMP for 48 h and then fixed. After permeabilization, samples were stained with Alexa 488-labeled Hsp90 antibody. In addition, after permeabilization, samples were incubated overnight with a MAG antibody or S100β antibody at 4°C. The cells were visualized using Alexa Fluor 488- or Cy3-conjugated donkey anti-mouse or anti-rabbit IgG, respectively. Cells were co-labeled with Hoechst dye. The results are representative of three experiments. **c** Rat primary SCs were treated with or without 500 μM db-cAMP for 48 hand the morphology was observed under an inverted microscope. Cell morphology was changed from an elongated bipolar shape to an epithelial type following treatment with 500 μM db-cAMP (×200). **d** Left panel: rat primary SCs were pretreated with 5 μM H-89 (PKA inhibitor) or 50 μM Rp-cAMPS (PKA inhibitor) for 1 h, followed by treatment with 500 μM db-cAMP for 48 h. Protein lysates were prepared and used for western blot analysis with the corresponding antibodies. β-actin was used as a loading control. The blot is representative of three separate experiments. Densitometry results are expressed above the bands. Right panel: Relative protein level compared to β-actin by densitometry was expressed as average ± SD (n = 3). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. **e** Left panel: rat primary SCs were transiently transfected using lipofectamine 3000 for 48 h with siRNAs against PKA Cα. A non-targeting control (NC) siRNA was used as the control. The cells were treated with db-cAMP for 48 h. The untreated cells served as controls. Protein lysates were prepared and used for western blot analysis with the corresponding antibodies. β-actin was used as a loading control. The blot is representative of three separate experiments. Densitometry results are expressed above the bands. Right panel: The relative protein level compared with β-actin determined via densitometry was expressed as an average ± SD (n = 3). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. **f** Left panel: rat primary SCs were treated with db-cAMP (500 μM), 6-Bnz-cAMP (500 μM) or 8-CPT-2Me-cAMP (500 μM) for 48 h. Protein lysates were prepared and used for western blot analysis with the corresponding antibodies. β-actin was used as a loading control. The blot is representative of three separate experiments. Densitometry results are expressed above the bands. Right panel: Relative protein level compared with β-actin by densitometry was expressed as average ± SD (n = 3). *$P < 0.05$, ***$P < 0.001$, NS: no significance.

### Statistical Analysis

Statistical analysis was performed using the SPSS 23.0 statistical package for Windows (SPSS, Chicago, IL, USA). Data are presented as mean ± standard deviation (SD) of at least three independent experiments. The Student's t test and one-way analysis of variance (ANOVA) were used to measure statistical significance. Statistical significance was defined as $P < 0.05$.

## Results

### Dibutyryl-cAMP (db-cAMP) Upregulates the Expression of Hsp90 via PKA Activation in Rat Primary SCs

cAMP is known as a differentiation-inducing signaling molecule in SCs [5, 6]. A recent report showed that PKA directly transactivates the ErbB2-Gab1 axis independent of NRG1 during SC differentiation [9]. Since Hsp90 is a chaperone for ErbB2 stabilization [15, 18], in this study, we examined whether cAMP induces Hsp90 to stabilize the ErbB2-Gab1 pathway in rat primary SCs. As shown in Figs. 1a and 2a, db-cAMP (cell-permeable analogue of cAMP) treatment increased the PKA activity (determined by western blot analysis of p-PKA substrate antibody) and the expression of Hsp90 with time in rat primary SCs. We also observed the increased expression of Hsp90 by db-cAMP treatment using immunofluorescence staining (Fig. 1b). In addition, to confirm the SC differentiation by db-cAMP, we examined the morphological changes and determined the expression of TFs (Oct6 and Krox20) controlling SC myelination, and myelin proteins by western blot analysis. Consistent with previous reports [26], the SC morphology was changed from a bipolar to an expanded shape similar to epithelium (Fig. 1c). We observed the upregulation of Oct6, Krox20, MPZ, and MAG, and the downregulation of c-jun by db-cAMP in rat primary SCs (Fig. 1a). We also determined the MAG expression via db-cAMP treatment using immunofluorescence staining (Fig. 1b). In addition, we evaluated whether cAMP enhances Hsp90 via PKA activation by using H-89 and Rp-cAMPS, which are selective inhibitors of PKA, and PKA Cα siRNA. Pretreatment with H-89, Rp-cAMPS, and PKA Cα siRNA significantly inhibited the PKA activity (determined by western blot analysis of p-PKA substrate antibody) (Fig. 2b–d) and the expression of Hsp90, Oct6, Krox20, MPZ, and MAG by db-cAMP (Fig. 1d, e). PKA and exchange proteins directly activated by cAMP (EPAC) are downstream targets of cAMP [27]. To determine whether either of these targets play a role in rat primary SC differentiation, the PKA-specific activator 6-Bnz-cAMP or the EPAC agonist (8-CPT-2Me-cAMP) was used. Rat primary SCs were treated with 6-Bnz-cAMP or 8-CPT-2Me-cAMP for 48 h. The results showed that 6-Bnz-cAMP induced the expression of Hsp90, Oct6, Krox20, MPZ, and MAG; however, 8-CPT-2Me-cAMP alone did not induce Hsp90, Oct6, Krox20, MPZ, and MAG in rat primary SCs (Fig. 1f). These results suggest that PKA may mediate the upregulation of Hsp90 by db-cAMP.

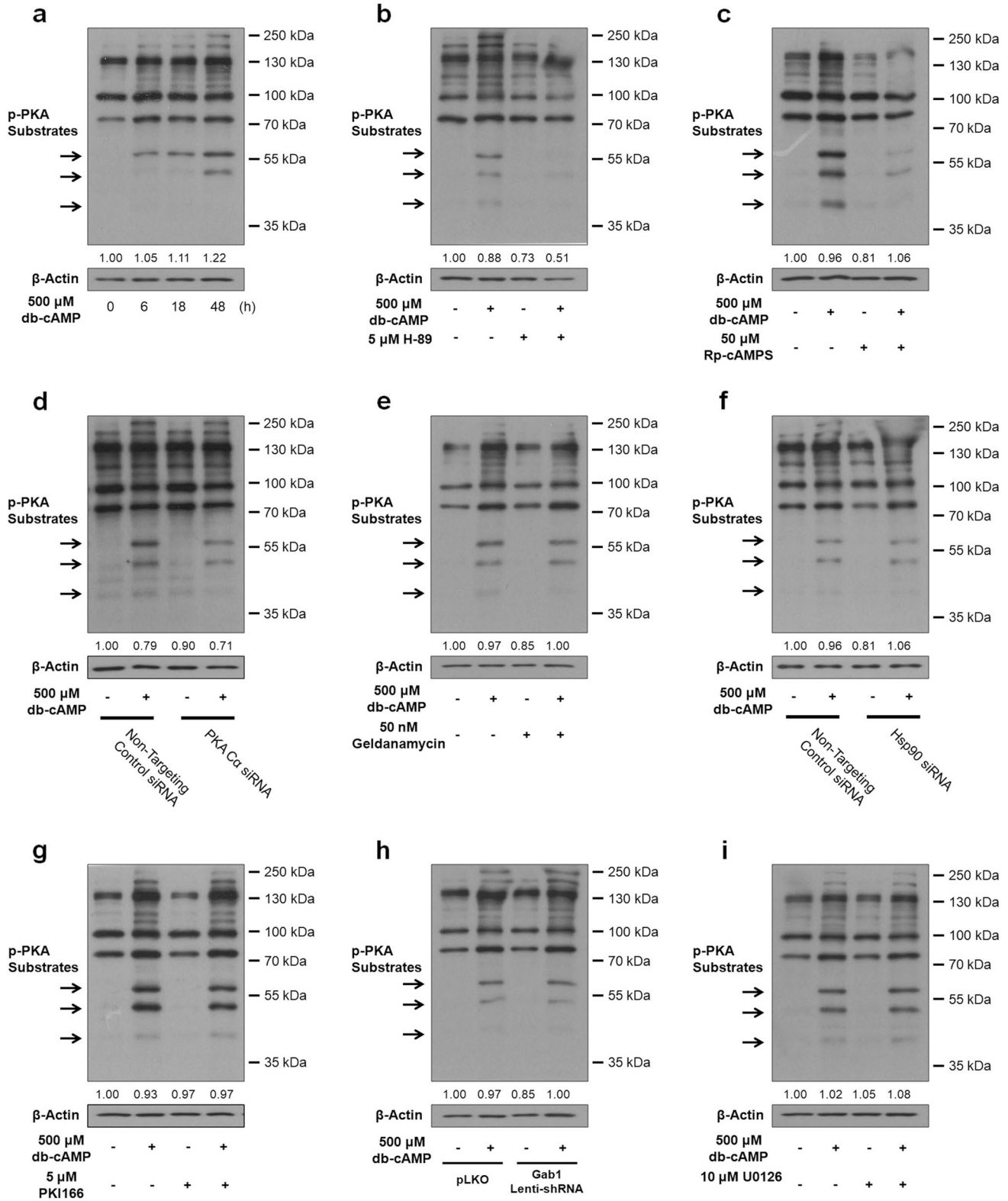



**Fig. 2** Change in PKA activity was determined by western blot analysis using antibodies targeting the p-PKA substrate. **a** db-cAMP activates PKA activity in a time-dependent manner. Immunoblots showing different amounts of p-PKA substrates. **b**–**d** Immunoblots show downregulation of the p-PKA substrate by H-89 (**b**), cAMPS-Rp (**c**), and PKA Cα siRNA (**d**). **e**, **f** GA (**e**) and Hsp90 siRNA (**f**) do not affect PKA activity **g** PKI166 does not affect PKA activity. **h** Gab1 shRNA does not affect PKA activity. **i** UO126 does not affect PKA activity. The blot is representative of three separate experiments.

## Geldanamycin, an Inhibitor of Hsp90, and Hsp90 siRNA Knockdown Inhibit cAMP-Induced Differentiation

It has been known that Hsp90 enhances protein stability by forming an ATP-dependent complex with various proteins including ErbB2, to prevent proteasome-dependent degradation [28, 29]. Due to the increased expression of Hsp90 during cAMP-induced differentiation, we analyzed the role of Hsp90 in cAMP-induced differentiation using geldanamycin (GA), a Hsp90 inhibitor. GA is a specific inhibitor binding to the ATP-binding site in the chaperone [30]. As shown in Fig. 3a, GA pretreatment decreased the expression of Oct6, Krox20, MPZ, and MAG following db-cAMP treatment. These results suggest that cAMP-induced differentiation may occur via Hsp90 upregulation and activation. We also found that db-cAMP-induced SC differentiation increased the levels of ErbB2 and p-ErbB2, and that GA totally suppressed the increased levels of ErbB2 and p-ErbB2 by db-cAMP. Further, GA did not affect db-cAMP-mediated PKA activity, which was determined by western blot analysis of p-PKA substrate antibody (Fig. 2e).

To further evaluate the role of Hsp90 in cAMP-induced differentiation, we elucidated the effects of siRNA-mediated knockdown of Hsp90aa and Hsp90ab on cAMP-induced differentiation in rat primary SCs. As shown in Fig. 3b, the downregulation of Hsp90 by siRNA knockdown was confirmed and the expression of ErbB2 as well as myelin proteins such as Oct6, Krox-20, MPZ and MAG by db-cAMP was reduced by Hsp90 siRNAs. However, the PKA activity was not affected by Hsp90 siRNA (Fig. 2f), suggesting that db-cAMP-induced PKA activation occurs upstream of Hsp90 upregulation. These results suggest that Hsp90 might be involved in cAMP-induced differentiation and that Hsp90-mediated stabilization of ErbB2 was associated with cAMP-induced differentiation.

## PKI166, an ErbB2 Inhibitor, Inhibits cAMP-Induced Differentiation

It has been recently reported that the elevation of cAMP in SCs transactivates the ErbB2-Gab1 pathway. Our findings suggest that Hsp90-mediated stabilization of ErbB2 may play a role in db-cAMP-induced SC differentiation. To determine whether ErbB2 transactivation was involved in cAMP-induced SC differentiation, rat primary SCs were pretreated with an ErbB2 inhibitor, PKI166 and treated with db-cAMP for 48 h, and the expression of p-PKA substrate, ErbB2, p-ErbB2, Hsp90, Oct6, Krox 20, MPZ and MAG was determined by western blot analysis. As shown in Fig. 4b, Hsp90, ErbB2, p-ErbB2, Oct6, Krox20 and myelin proteins were stimulated by db-cAMP, and inhibited by PKI166 treatment. In addition, the PKA activity was not affected by PKI166 treatment (Fig. 2g), suggesting that db-cAMP-induced PKA activation occurs upstream of ErbB2 activation. These results are similar to those of GA treatment and Hsp90 siRNA transfection (Fig. 3). In addition, the tyrosine phosphorylation of Gab1, which is the downstream mediator of ErbB2 in myelination [13] following db-cAMP treatment, was also suppressed by PKI166. These data suggest that db-cAMP stabilizes ErbB2 via Hsp90 induction and that increased ErbB2 levels contribute to SC differentiation via Gab1 activation.

## Knockdown of Gab1 Inhibits cAMP-Induced Differentiation in Rat Primary SCs

As shown in Fig. 4a, p-Gab1 expression showed a time-dependent increase following db-cAMP treatment of rat primary SCs. Thus, we evaluated whether Gab1 activation was required for cAMP-induced differentiation using Gab1 shRNA transfection. As shown in Figs. 5a and 2h, Gab1 shRNA knockdown inhibited the expression of Hsp90, ErbB2, Oct6, Krox20 and myelin proteins; however, it did not inhibit the expression of p-ErbB2 and p-PKA substrate by db-cAMP. These results suggest that Gab1 activation occurs downstream of PKA and ErbB2 activation, and Gab1 activation mediates the db-cAMP-induced SC differentiation.

## Gab1 Dissociates from Hsp90 and Translocates into Nucleus with ERK1/2 During db-cAMP-Induced Differentiation

The foregoing results suggest that Hsp90 is required for the cAMP-induced differentiation. However, the underlying molecular mechanisms are clearly undefined. We investigated the interaction between Hsp90 and Gab1 during db-cAMP-induced differentiation via immunoprecipitation. In Gab1 immunoprecipitates, the reaction with Hsp90 antibody revealed Gab1 and Hsp90 interaction in untreated cells and dissociation of Gab1 from Hsp90 in db-cAMP-treated rat primary SCs (Fig. 5b). We also performed immunofluorescence staining to determine the location of Hsp90 and Gab1 during db-cAMP-induced differentiation. Very interestingly, in unstimulated cells, both Hsp90 and Gab1 were localized in the cytoplasm and Gab1 moved into the nucleus following db-cAMP treatment while Hsp90 remained in the cytosol (Fig. 5c). To investigate the role of Hsp90 in Gab1 activation and nuclear translocation during cAMP-induced differentiation, GA pretreatment and Hsp90 siRNA knockdown experiments were performed before db-cAMP treatment of rat primary SCs. As shown in Figs. 2a, b, 4d, the activation and the nuclear translocation of Gab1 by db-cAMP was inhibited by GA and Hsp90 siRNA transfection, respectively. These results suggest that Hsp90 is required for the activation and

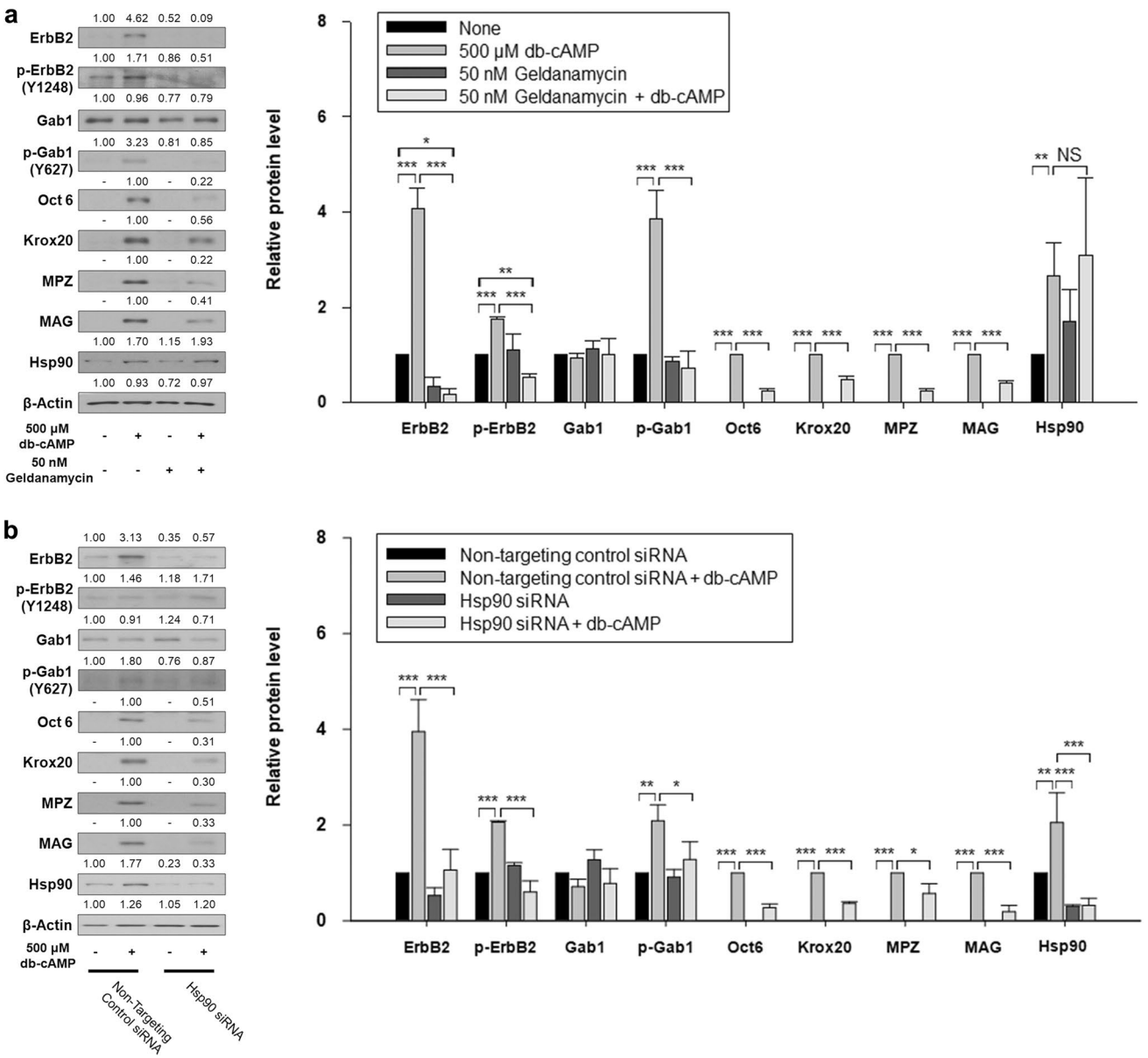



**Fig. 3** Hsp90 inhibition by chemical inhibitor and knockdown of HSP90 inhibited db-cAMP-induced differentiation in rat primary SCs. **a** Rat primary SCs were pretreated with 50 nM GA (geldanamycin, hsp90 inhibitor) for 1 h, followed by treatment with 500 μM db-cAMP for 48 h. Protein lysates were prepared and analyzed via western blot with the corresponding antibodies. β-actin was used as a loading control. The blot is representative of three separate experiments. Densitometry results are expressed above the bands. Right panel: Relative protein level compared with β-actin by densitometry was expressed as average ± SD (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. **b** Left panel: rat primary SCs were transiently transfected by lipofectamine 3000 for 48 h with siRNAs against Hsp90aa1 and Hsp90ab1. A non-targeting control (NC) siRNA was used as control. The cells were untreated (control) or treated with db-cAMP for 48 h. Protein lysates were prepared and used for western blot analysis with the corresponding antibodies. β-actin was used as a loading control. The blot is representative of three separate experiments. Densitometry results are expressed above the bands. Right panel: Relative protein level compared with β-actin by densitometry was expressed as average ± SD (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

the nuclear translocation of Gab1 during db-cAMP-induced differentiation.

A previous study showed that ERK activation occurred downstream of Gab1 activation [13]. In this study, we observed the activation of ERK, evidenced by increased p-ERK expression and nuclear translocation of ERK after treatment with db-cAMP in rat primary SCs (Fig. 6a, b). To determine whether the activation of ERK was required for db-cAMP-induced differentiation, the cells were pretreated with ERK and MEK inhibitors for 1 h before db-cAMP treatment for 48 h, and the expression of p-PKA substrate, Oct6, Krox20, MPZ, and MAG was analyzed by

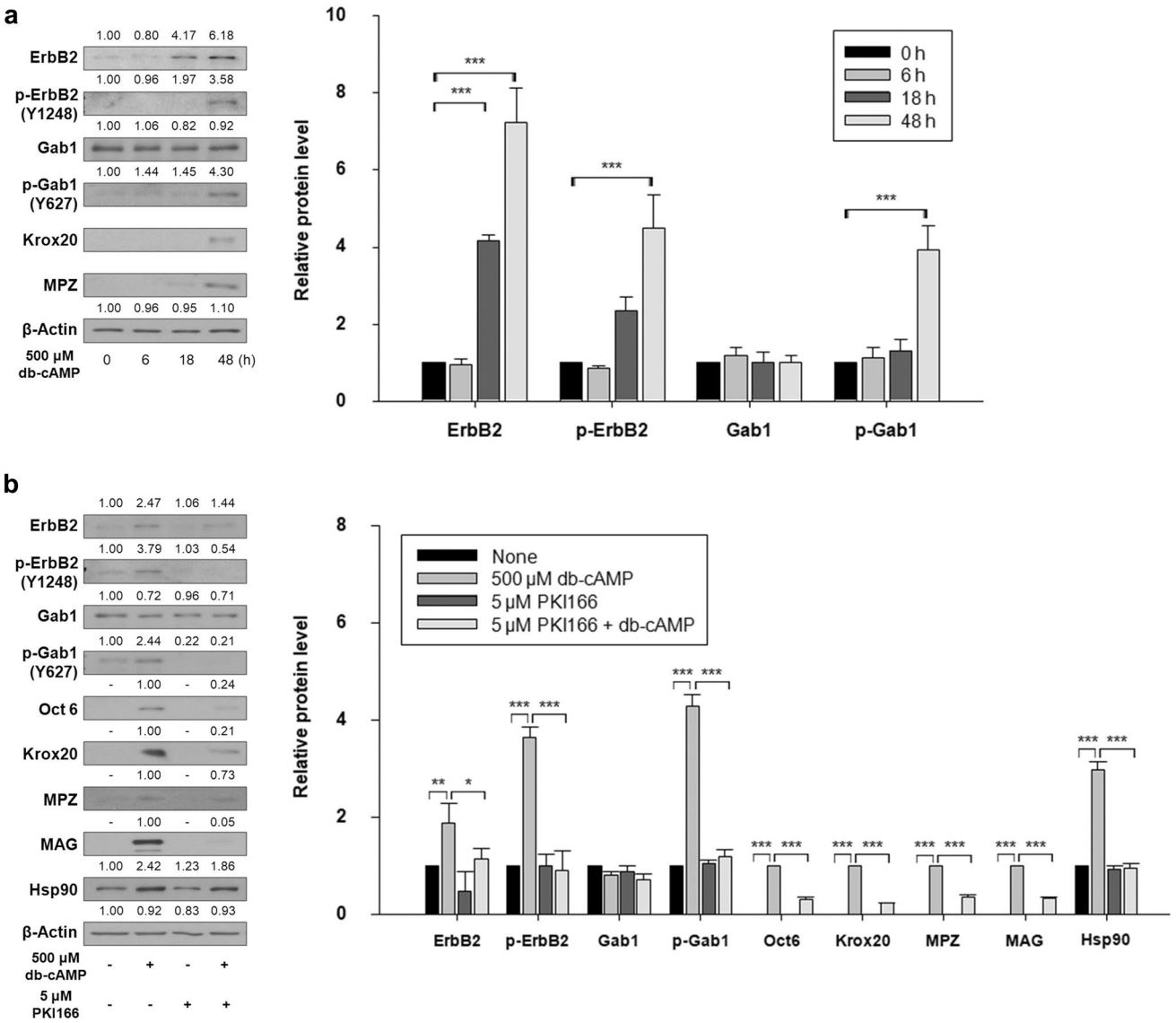
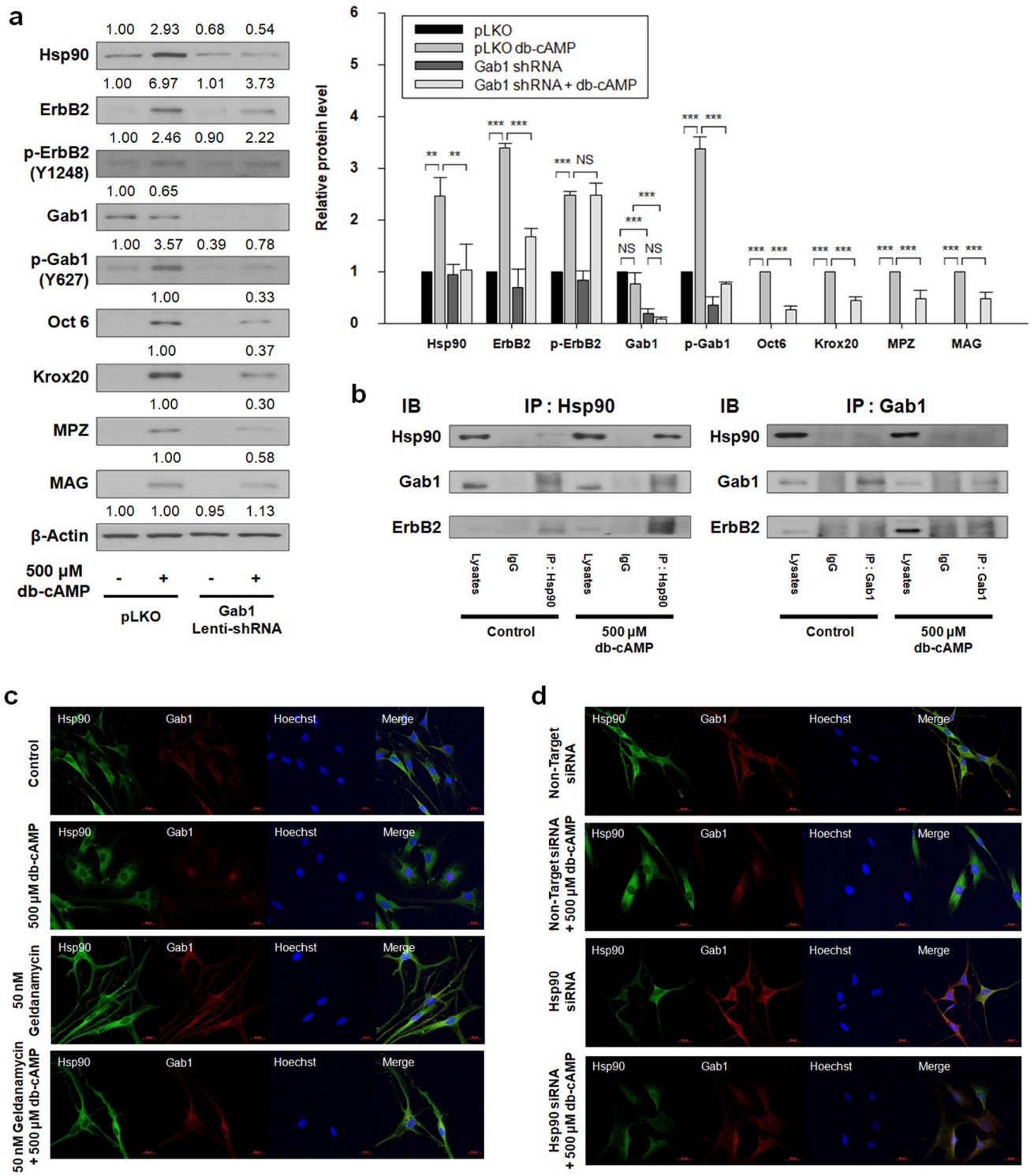



**Fig. 4** db-cAMP transactivates ErbB2 and ErbB2 activation is required for db-cAMP-induced differentiation in rat primary SCs. **a** Left panel: ratprimary SCs were treated with 500 μM db-cAMP for indicated times. Protein lysates were prepared and used for western blot analysis with the corresponding antibodies. β-actin was used as a loading control. The blot is representative of three separate experiments. Densitometry results are expressed above the bands. Right panel: Relative protein level compared with β-actin by densitometry was expressed as average ± SD (n = 3). ***$P < 0.001$. **b** Left panel: rat primary SCs were pretreated with 5 μM PKI166 (ErbB2 inhibitor) for 1 h, followed by treatment with 500 μM db-cAMP for 48 h. Protein lysates were prepared and used for western blot analysis with the corresponding antibodies. β-actin was used as a loading control. The blot is representative of three separate experiments. Densitometry results are expressed above the bands. Right panel: Relative protein level compared with β-actin by densitometry was expressed as average ± SD (n = 3). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

western blot analysis. As shown in Fig. 6c, the expression of Oct6, Krox20, MPZ, and MAG was inhibited by inhibitors of MEK and ERK. However, the db-cAMP-induced expression of p-PKA substrate was not inhibited by MEK inhibitor (Fig. 2i), suggesting that PKA activation by db-cAMP occurs upstream of ERK activation. These results suggest that ERK activation was involved in db-cAMP-induced differentiation. To further elucidate the role of Hsp90 and Gab1 in nuclear translocation of ERK during db-cAMP-induced differentiation, we performed immunofluorescence staining with Hsp90, Gab1 and ERK antibodies in Hsp90 siRNA- and Gab1 shRNA-transfected rat primary SCs. The results showed that the nuclear translocation of ERK by db-cAMP was inhibited by Hsp90 siRNA and Gab1 shRNA transfection (Fig. 6d, e). These results indicate that Hsp90 and Gab1 are required for the nuclear translocation of ERK during cAMP-induced differentiation.




## Discussion

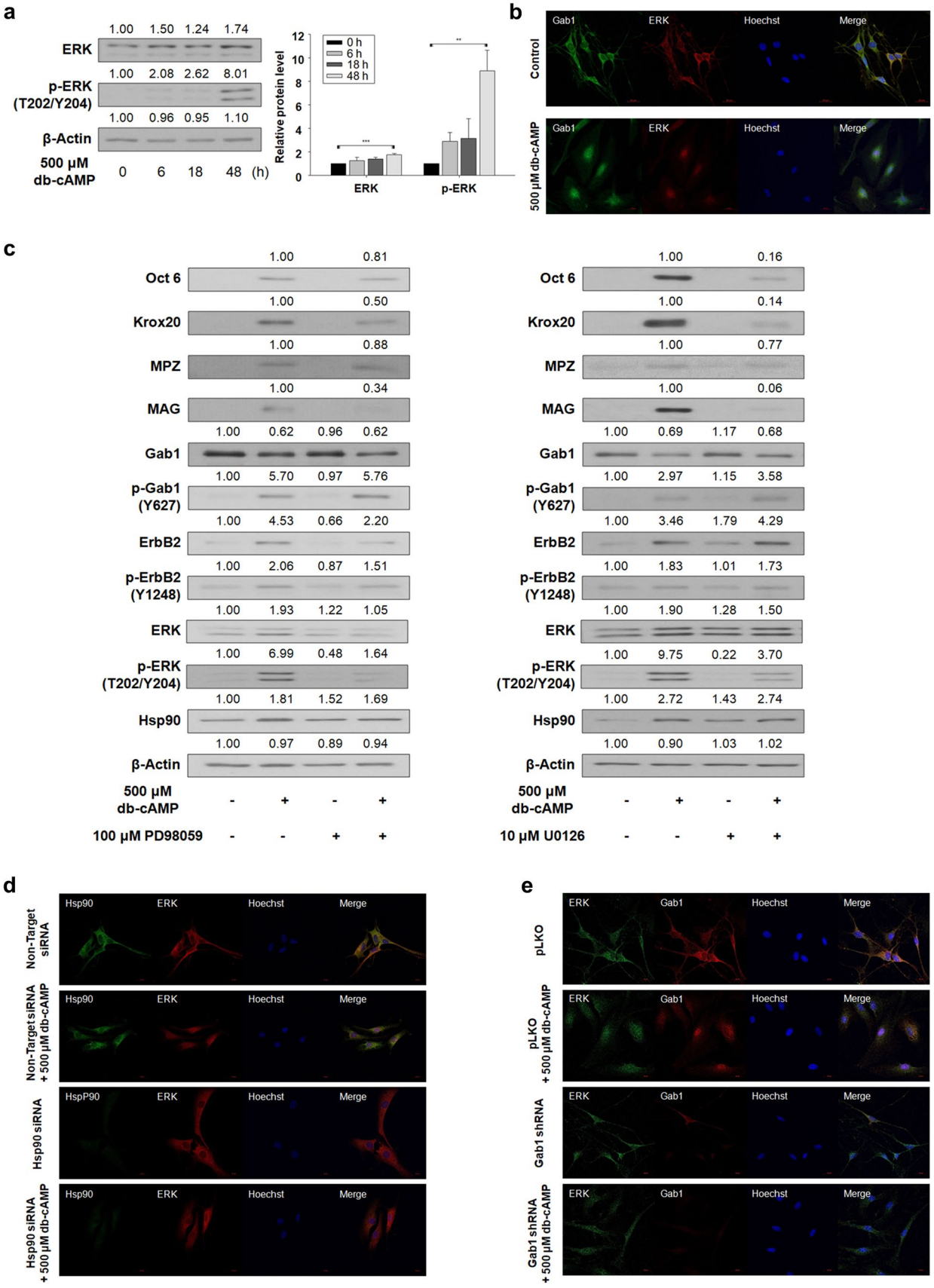
cAMP has been known to act as an instructive signal for differentiation of rat SCs into myelinating phenotypes [5, 6]. However, the effect of cAMP on cultured SCs is very complex depending on the concentration of cAMP, the presence of growth factors and/or serum, and the source of SCs such as mouse or rat [31–37]. A recent study demonstrated that PKA directly transactivated the ErbB2-Gab1 axis independent of NRG1 during SC differentiation [12]. However, the mechanisms underlying the NRG1-independent PKA transactivation of ErbB2-Gab1 axis remain unknown. Based on

◂**Fig. 5** Gab1 activation is required for db-cAMP-induced differentiation of rat primary SCs. **a** Left panel: rat primary SCs were infected with lentiviral transduction particles expressing shRNA for Gab1 or with empty vector control transduction particles (pLKO) and incubated in DMEM medium with 500 μM db-cAMP for 48 h. Protein lysates were prepared and used for western blot analysis with the corresponding antibodies. β-actin was used as a loading control. The blot is representative of three separate experiments. Densitometry results are expressed above the bands. Right panel: Relative protein level compared with β-actin by densitometry was expressed as average ± SD (n = 3). $^{**}P < 0.01$, $^{***}P < 0.001$, *NS* no significance. **b** Rat primary SCs were treated for 48 h with or without 500 μM db-cAMP, and lysed in RIPA buffer. Hsp90 proteins (Left) and Gab1 proteins (right) were immunoprecipitated by incubating the cell lysates with Hsp90 antibody or Gab1 antibody, followed by protein G Plus-agarose beads with anti-mouse IgG or anti-rabbit IgG, respectively. Immunoprecipitated proteins were solubilized in SDS sample buffer and separated by SDS-PAGE. Blots were probed with anti-Hsp90, anti-Gab1, or anti-ErbB2 antibodies. **c** Rat primary SCs were pretreated with 50 nM GA (geldanamycin, hsp90 inhibitor) for 1 h, followed by treatment with 500 μM db-cAMP for 48 h and then fixed. After permeabilization, samples were stained with anti-Hsp90 antibody and Alexa 488-conjugated donkey anti-mouse IgG or anti-Gab1 antibody and Cy3-conjugated donkey anti-rabbit IgG. Cells are co-labeled with Hoechst dye. The results are representative of three experiments. **d** Rat primary SCs were transiently transfected by lipofectamine 3000 for 48 h with siRNAs against Hsp90aa1 and Hsp90ab1. A non-target control (NC) siRNA was used as the control. After transfection, cells were untreated (control) or treated with db-cAMP for 48 h and then fixed. After permeabilization, samples were stained with anti-Hsp90 antibody and Alexa 488-conjugated donkey anti-mouse IgG or anti-Gab1 antibody and Cy3-conjugated donkey anti-rabbit IgG. Nuclei were counterstained with Hoechst. The results are representative of three experiments

the finding that Hsp90 binds to ErbB2 and thereby stabilizes ErbB2 [15, 18], we hypothesized that cAMP activates PKA, which then upregulates Hsp90 and stabilizes ErbB2, resulting in SC differentiation. Here, we demonstrate that db-cAMP upregulates Hsp90 and ErbB2 in a time-dependent manner and induces the expression of myelin-related TFs (Oct6 and Krox20) and myelin proteins (MPZ and MAG). Addition of PKA inhibitor H-89, Rp-cAMP or transfection of PKA Cα siRNA before treatment with db-cAMP blocked the upregulation of Hsp90 and ErbB2 and the expression of myelin-related genes. Hsp90 is a molecular chaperone promoting the stability and function of many signaling proteins [28, 29]. Fregien et al. reported that db-cAMP increased HER2 and HER3 expression without increasing their mRNAs in human SCs and H-89 inhibited the increased expression of HER2 and HER3 [31]. They suggested that the increased expression of HER2 and HER3 by db-cAMP enhanced the proliferative effect of neuregulin by db-cAMP in human SCs [38]. However, until now, no study investigated the role of Hsp90 in cAMP-induced SC differentiation. Our findings suggest that PKA-mediated Hsp90 upregulation is required for db-cAMP-induced differentiation, because GA pretreatment and Hsp90 siRNA knockdown reversed the increased expression of ErbB2 and myelin-related genes by db-cAMP. To our knowledge, this is the first report showing that Hsp90 is required for db-cAMP-induced SC differentiation. We also observed that PKA specific agonist 6-Bnz-cAMP induced Hsp90 upregulation and myelin-related genes, unlike EPAC-specific agonist 8-CPT-2Me-cAMP. These results are contradictory to those of Bacallao's study [6]. They reported that PKA was involved in cAMP-induced proliferation and EPAC mediated the cAMP-induced differentiation [6]. These discrepancies can be explained by different cells and culture conditions. Further studies to explain these discrepancies are needed.

In accordance with a previous report [12], we observed the increased expression of p-ErbB2 and p-Gab1, which are important molecules involved in NRG1-induced myelination, following db-cAMP treatment without NRG1 in rat primary SCs. It was shown that Gab1 was transiently activated by NRG1 treatment in rat primary SCs [13]. In contrast, Gab1 activation was delayed and sustained after db-cAMP treatment of rat primary SCs. These results suggest that the delayed and sustained activation of Gab1 might play a role in db-cAMP-induced differentiation. Until now, no evidence suggested any molecular interactions between Hsp90 and Gab1 in rat primary SCs. Herein, we found that Gab1 interacted with Hsp90 in the absence of db-cAMP; however, Gab1 was dissociated from Hsp90 and interacted with ErbB2 following exposure to db-cAMP in rat primary SCs. Surprisingly, we observed the nuclear translocation of Gab1 by db-cAMP in rat primary SCs, unlike NRG1-induced Gab1 activation. Gab1 is known to exhibit a nuclear localization signal at its N-terminal [39], and thus, we focused on the role of Gab1 nuclear translocation by db-cAMP during the persistent activation of Gab1.

ERK is known as a downstream molecule of Gab1 in the NRG1 pathway [13]. Shin et al. showed that ERK activation was inhibited in Gab1 SC knockout mice; however, they did not observe any nuclear translocation of Gab1 and ERK [13]. We clearly found the activation and nuclear translocation of ERK by db-cAMP in rat primary SCs. In addition, ERK inhibitor PD98059 and MEK inhibitor U0126 inhibited the expression of myelin-related genes by db-cAMP. These results indicate that ERK activation is also required for db-cAMP-induced SC differentiation. To determine whether Hsp90 was required for the sustained Gab1/ERK activation and their nuclear translocation during db-cAMP-induced differentiation, GA treatment and Hsp90 siRNA knockdown experiments were performed before treatment with db-cAMP. Very interestingly, GA pretreatment and Hsp90 siRNA inhibited the activation and nuclear translocation of Gab1 and ERK suggesting that Hsp90 may play a role in the activation and nuclear translocation of Gab1 and ERK following db-cAMP treatment. To further elucidate the role of activation and nuclear translocation of Gab1 in db-cAMP-induced SC differentiation, Gab1 shRNA knockdown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p-ERK (T202/Y204)
β-Actin
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Relative protein level
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18 h
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p-ERK
b
Gab1
ERK
Hoechst
Merge
Control
500 μM db-cAMP
c
Oct 6
Krox20
MPZ
MAG
Gab1
p-Gab1 (Y627)
ErbB2
p-ErbB2 (Y1248)
ERK
p-ERK (T202/Y204)
Hsp90
β-Actin
500 μM db-cAMP
100 μM PD98059
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◂**Fig. 6** Hsp90 is involved in the activation and translocation of Gab1/ERK during db-cAMP-induced differentiation in rat primary SCs. **a** Left panel: Rat primary SCs were treated with 500 μM db-cAMP for indicated times. Protein lysates were prepared and used in western blot analysis with anti-ERK or anti-p-ERK antibodies. β-actin was used as a loading control. The blot is representative of three separate experiments. Densitometry results are expressed above the bands. Right panel: Relative protein level compared with β-actin by densitometry was expressed as average ± SD (n = 3). $^{**}P < 0.01$, $^{***}P < 0.001$. **b** Rat primary SCs were treated with db-cAMP (500 μM) for 48 h and then fixed. After permeabilization, samples were stained with anti-Gab1 antibody and Alexa 488-conjugated donkey anti-rabbit IgG or anti-ERK antibody and Cy3-conjugated donkey anti-mouse IgG, respectively. Nuclei were counterstained with Hoechst. The results are representative of three experiments. **c** Rat primary SCs were pretreated with PD98059 (100 μM; ERK inhibitor) for 1 h (left panel) or U0126 (10 μM; MEK inhibitor) for 1 h (right panel), respectively, followed by treatment with 500 μM db-cAMP for 48 h. Protein lysates were prepared and used for western blot analysis with the corresponding antibodies. β-actin was used as a loading control. The blot is representative of three separate experiments. Densitometry results are expressed above the bands. **d** Rat primary SCs were transiently transfected with lipofectamine 3000 for 48 h with siRNAs against Hsp90aa1 and Hsp90ab1. A non-target control (NC) siRNA was used as control. The cells were untreated (control) or treated with db-cAMP for 48 h and then fixed. After permeabilization, samples were stained with anti-Hsp90 antibody and Alexa 488-conjugated donkey anti-rabbit IgG or anti-ERK antibody and Cy3-conjugated donkey anti-mouse IgG, respectively. Nuclei were counterstained with Hoechst. The results are representative of three experiments. **e** Rat primary SCs were infected with lentiviral transduction particles expressing shRNAi for Gab1 or with empty vector control transduction particles (pLKO) and incubated in DMEM medium with 500 μM db-cAMP for 48 h, and then fixed. After permeabilization, samples were stained with anti-ERK antibody, Alexa 488-conjugated donkey anti-mouse IgG or anti-Gab1 antibody, and Cy3-conjugated donkey anti-rabbit IgG, respectively. Nuclei were counterstained with Hoechst. The results are representative of three experiments

experiment was performed. Gab1 knockdown inhibited the activation and nuclear translocation of ERK and the expression of myelin-related genes by db-cAMP. Therefore, the tyrosine phosphorylation of Gab1 by ErbB2 transactivation appears to accompany ERK translocation into nucleus in the cAMP-induced differentiation. It has been known that Gab is required for nuclear translocation of ERK following exposure to 10% serum in bovine aortic endothelial cells and Chinese hamster ovary cells [39]. Our results are consistent with that study and the nuclear localization of Gab1/ERK may play a critical role in the transcriptional activation of myelin gene expression via cAMP elevation.

Based on our observations, we propose a model to explain the NRG1-independent mechanism of cAMP-induced transactivation of ErbB2/Gab1/ERK pathway in rat primary SCs (Fig. 7). Although we provided evidence suggesting that db-cAMP induces SC differentiation via upregulation of Hsp90 stabilizing ErbB2 resulting in the activation and nuclear translocation of Gab1/ERK in rat primary SCs, the detailed molecular mechanism of db-cAMP-induced differentiation is still unknown, especially the activation of ErbB2 by db-cAMP. We propose three possibilities underlying cAMP-mediated activation of ErbB2 in rat primary SCs. First, the activation of tyrosine kinase ligand other than NRG1 via upregulation of Hsp90 by db-cAMP may contribute to the activation of ErbB2 by db-cAMP. Second, db-cAMP may induce the secretion of NRG1-like tyrosine kinase to activate ErbB2. Third, the phosphorylation of ErbB3 by db-cAMP may enhance ErbB2 autophosphorylation. It will be very interesting to evaluate these possibilities in future studies to determine the molecular mechanisms of peripheral nerve myelination.

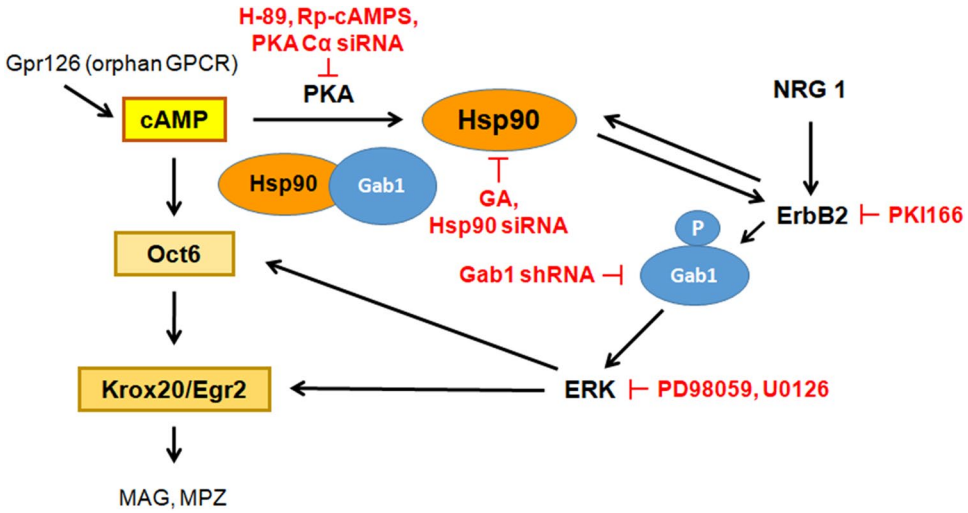


**Fig. 7** The hypothetical molecular mechanism of cAMP-mediated transactivation of ErbB2/Gab1/ERK pathway in rat primary SCs. cAMP induces SC differentiation via upregulation of Hsp90 by PKA followed by activation and nuclear translocation of Gab1/ERK

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## Compliance with Ethical Standards

**Conflict of interest** The authors have no potential conflicts of interest to disclose.

## References

1. Jessen KR, Mirsky R (2005) The origin and development of glial cells in peripheral nerves. Nat Rev Neurosci 6:671–682
2. Svaren J, Meijer D (2008) The molecular machinery of myelin gene transcription in Schwann cells. Glia 56:1541–1551
3. Salzer JL (2015) Schwann cell myelination. Cold Spring Harb Perspect Biol 7:a020529
4. Taveggia C, Feltri ML, Wrabetz L (2010) Signals to promote myelin formation and repair. Nat Rev Neurol 6:276–287
5. Jessen KR, Mirsky R, Morgan L (1991) Role of cyclic AMP and proliferation controls in Schwann cell differentiation. Ann NY Acad Sci 633:78–89
6. Bacallao K, Monje PV (2013) Opposing roles of PKA and EPAC in the cAMP-dependent regulation of Schwann cell proliferation and differentiation. PLoS ONE 8:e82354
7. Howe DG, McCarthy KD (2000) Retroviral inhibition of cAMP-dependent protein kinase inhibits myelination but not Schwann cell mitosis stimulated by interaction with neurons. J Neurosci 20:3513–3521
8. Newbern J, Birchmeier C (2010) Nrg1/ErbB signaling networks in Schwann cell development and myelination. Semin Cell Dev Biol 21:922–928
9. Traveggia C, Zanazzi G, Petrylak A, Yano H, Rosenbluth J, Einheber S, Xu X, Esper RM, Loeb JA, Shrager P, Chao MV, Falls DL, Role L, Salzer JL (2005) Neuregulin-1 type III determines the ensheathment fate of axons. Neuron 47:681–694
10. Topilko P, Schneider-Maunoury S, Levi G, Baron-Van Evercooren A, Chen-noufi AB, Seitanidou T, Babinet C, Charnay P (1994) Krox-20 controls myelination in the peripheral nervous system. Nature 371:796–799
11. He Y, Kim JY, Dupree J, Tewari A, Melendez-Vasquez C, Svaren J, Casaccia P (2010) Yy1 as molecular link between neuregulin and transcriptional modulation of peripheral myelination. Nat Neurosci 13:1472–1480
12. Ghidinelli M, Poitelon Y, Shin YK, Ameroso D, Williamson C, Ferri C, Pellegatta M, Espino K, Mogha A, Monk K, Podini P, Taveggia C, Nave KA, Wrabetz L, Park HT, Feltri ML (2017) Laminin 211 inhibits protein kinase A in Schwann cells to modulate neuregulin 1 type III-driven myelination. PLoS Biol 15:e2001408
13. Shin YK, Jang SY, Park SY, Park JY, Kim JK, Kim JP, Suh DJ, Lee HJ, Park HT (2014) Grb2-associated binder-1 is required for neuregulin-1-induced peripheral nerve myelination. J Neurosci 34:7657–7662
14. Sidera K, Samiotaki M, Yfanti E, Panayotou G, Patsavoudi E (2004) Involvement of cell surface HSP90 in cell migration reveals a novel role in the developing nervous system. J Biol Chem 279:45379–45388
15. Taipale M, Jarosz DF, Lindquist S (2010) HSP90 at the hub of protein homeostasis: emerging mechanistic insights. Nat Rev Mol Cell Biol 11:518–528
16. Sreedhar AS, Kalmár E, Csermely P, Shen YF (2004) Hsp90 isoforms: functions, expression and clinical importance. FEBS Lett 562:11–15
17. Citri A, Gan J, Mosesson Y, Vereb G, Szollosi J, Yarden Y (2004) Hsp90 restrains ErbB-2/HER2 signalling by limiting heterodimer formation. EMBO Rep 5:1165–1170
18. Sidera K, Gaitanou M, Stellas D, Matsas R, Patsavoudi E (2008) A critical role for HSP90 in cancer cell invasion involves interaction with the extracellular domain of HER-2. J Biol Chem 283:2031–2041
19. Dote H, Cerna D, Burgan WE, Camphausen K, Tofilon PJ (2005) ErbB3 expression predicts tumor cell radiosensitization induced by Hsp90 inhibition. Cancer Res 65:6967–6975
20. Pratt WB, Morishima Y, Osawa Y (2008) The Hsp90 chaperone machinery regulates signalling by modulating ligand binding clefts. J Biol Chem 283:22885–22889
21. Rangaraju S, Madorsky I, Pileggi JG, Kamal A, Notterpek L (2008) Pharmacological induction of the heat shock response improves myelination in a neuropathic model. Neurobiol Dis 32:105–115
22. Urban MJ, Li C, Yu C, Lu Y, Krise JM, Mclntosh MP, Rajewski RA, Blagg BS, Dobrowsky RT (2010) Inhibiting heat-shock protein 90 reverses sensory hypoalgesia in diabetic mice. ASN Neuro 2:e00040
23. Li C, Ma J, Zhao H, Blagg BS, Dobrowsky RT (2012) Induction of heat shock protein 70 (Hsp70) prevents neuregulin-induced demyelination by enhancing the proteasomal clearance of c-Jun. ASN Neuro 4:e00102
24. Shin YK, Jang SY, Lee HK, Jung J, Suh DJ, Seo SY, Park HT (2010) Pathological adaptive responses of Schwann cells to endoplasmic reticulum stress in bortezomib-induced paripheral neuropathy. Glia 58:1961–1976
25. Shin SW, Seo CY, Han H, Han JY, Jeong JS, Kwak JY, Park JI (2009) 15d-$PGJ_2$ induces apoptosis by reactive oxygen species-mediated inactivation of Akt in leukemia and colorectal cancer cells and shows in vivo antitumor activity. Clin Cancer Res 15:5414–5425
26. Monje PV, Rendon S, Athauda G, Bates M, Wood PM, Bunje MB (2009) Non-antagonistic relationship between mitogenic factors and cAMP in adult Schwann cell redifferentiation. Glia 57:947–961
27. Bos JL (2003) Epac: A new cAMP target and new avenues in cAMP research. Nat Rev Mol Cell Biol 4:733–738
28. Xu M, Mimnaugh E, Rosser MF, Nichchita C, Marcu M, Yarden Y, Neckers L (2001) Sensitivity of mature Erbb2 to geldanamycin is conferred by its kinases domain and is mediated by the chperone protein Hsp90. J Biol Chem 276:3702–3708
29. Xu W, Mimnaugh EG, Kim JS, Trepel JB, Neckers LM (2002) Hsp90, not Grp94, regulates the intracellular trafficking and stability of nascent ErbB2. Cell Stress Chaperones 7:91–96
30. Grenert JP, Sullivan WP, Fadden P, Haystead TA, Clark J, Mimnaugh E, Krutzsch H, Ochel HJ, Schulte TW, Sausville E, Neckers LM, Toft DO (1997) The amino-terminal domain of heat shock protein 90 (hsp90) that binds geladanamycin is an ATP-ADP swich domain that regulates hsp90 conformation. J Biol Chem 272:23843–23850
31. Yamada H, Komiyada A, Suzuki K (1995) Schwann cell responses to forskolin and cyclic AMP analogues: comparative study of mouse and rat Schwann cells. Brain Res 681:97–104
32. Morgan L, Jessen KR, Mirsky R (1991) The effects of cAMP on differentiation of cultured Schwann cells: progression from an early phenotype (04+) to a myelin phenotype (P0+, GFAP-,

N-CAM-, NGF-receptor-) depends on growth inhibition. J Cell Biol 112:457–467
33. Monje PV, Bartlett Bunge M, Wood PM (2006) Cyclic AMP synergistically enhances neuregulin-dependent ERK and Akt activation and cell cycle progression in Schwann cells. Glia 53:649–659
34. Kim HA, DeClue JE, Ratner N (1997) cAMP-dependent protein kinase A is required for Schwann cell growth: interactions between the cAMP and neuregulin/tyrosine kinase pathways. J Neurosci Res 49:236–247
35. Stewart HJ, Eccleston PA, Jessen KR, Mirsky R (1991) Interaction between cAMP elevation, identified growth factors, and serum components in regulating Schwann cell growth. J Neurosci Res 30:346–352
36. Rahmatullah M, Schroering A, Rothblum K, Stahl RC, Urban B, Carey DJ (1998) Synergistic regulation of Schwann cell proliferation by heregulin and forskolin. Mol Cell Biol 18:6245–6252
37. Arthur-Farraj P, Wanek K, Hantke J, Davis CM, Jayakar A, Parkinson DB, Mirsky R, Jessen KR (2011) Mouse schwann cells need both NRG1 and cyclic AMP to myelinate. Glia 59:720–733
38. Fregien NL, White LA, Bunge MB, Wood PM (2005) Forskolin increases neuregulin receptors in human Schwann cells without increasing receptor mRNA. Glia 49:24–35
39. Osawa M, Itoh S, Ohta S, Huang Q, Berk BC, Mamarosh NL, Che W, Ding B, Yan C, Abe J (2004) ERK1/2 associates with the c-Met-binding domain of growth factor receptor-bound protein 1 (Grb2)-associated binder-1 (Gab1): role in ERK1/2 and early growth response factor-1 (Egr-1) nuclear accumulation. J Biol Chem 279:29691–29699