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Methylcobalamin promotes proliferation and migration and inhibits apoptosis of C2C12 cells via the Erk1/2 signaling pathway

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ABSTRACT

Methylcobalamin (MeCbl) is a vitamin B12 analog that has some positive effects on peripheral nervous disorders. Although some previous studies revealed the effects of MeCbl on neurons, its effect on the muscle, which is the final target of motoneuron axons, remains to be elucidated. This study aimed to determine the effect of MeCbl on the muscle. We found that MeCbl promoted the proliferation and migration of C2C12 myoblasts *in vitro* and that these effects are mediated by the Erk1/2 signaling pathway without affecting the activity of the Akt signaling pathway. We also demonstrated that MeCbl inhibits C2C12 cell apoptosis during differentiation. Our results suggest that MeCbl has beneficial effects on the muscle *in vitro*. MeCbl administration may provide a novel therapeutic approach for muscle injury or degenerating muscle after denervation.

1. Introduction

Vitamin B12 is important for maintaining the normal function of the nervous system, and its deficiency causes a systemic neuropathy called subacute combined degeneration of the spinal cord [1]. Methylcobalamin (MeCbl) is one of the vitamin B12 analogs. We previously reported that MeCbl is the most effective vitamin B12 analog for neurite outgrowth in cerebellar granule neurons and dorsal root ganglion neurons *in vitro* [2] and it increases the mammalian target of rapamycin activity via Akt activation [3]. MeCbl promoted nerve regeneration in *in vivo* nervous disorder models such as those of rat sciatic nerve injury [2,4], streptozotocin-diabetic rats [5], and experimental acrylamide neuropathy [6]. Only one report has described the effect of MeCbl on the neuromuscular junction and indicated that MeCbl promotes the regeneration of motor nerve terminals, a component of the neuromuscular junction, degenerating in the anterior gracile muscle of the gracile axonal dystrophy mutant mouse [4]. Although these previous reports established the effects of MeCbl on neurons and the neuromuscular junction both *in vitro* and *in vivo*, there is no report concerning its effects on the muscle, which is the final target of motoneuron axons. Although MeCbl probably influences not only the nervous system but also the muscle tissue, its effects on the muscle remains unknown.

In the adult skeletal muscle, myogenic satellite cells are quiescent and located between the sarcolemma and basal lamina. In response to stimuli such as myotrauma or nerve injury, myogenic satellite cells become activated, proliferate, and express myogenic markers. Eventually, these cells fuse with existing muscle fibers or fuse together to form new myofibers [7]. Some reports have indicated that transplanting primary satellite cells or injecting fibroblast growth factor-2, which upregulates both myoblast proliferation and fusion, improves the properties of reinnervated skeletal muscles [8,9]. These results suggest that an increase in the number of satellite cells is important as the first step for the regeneration of the damaged skeletal muscle and affects its properties. The Erk1/2 signaling pathway is an important factor involved in myogenic satellite cell proliferation [10,11].

In this study, we demonstrate novel effects of MeCbl on C2C12 myoblasts. We found that MeCbl promotes the proliferation and migration of C2C12 myoblasts via the Erk1/2 signaling pathway. We also exhibited that MeCbl inhibits C2C12 cell apoptosis. Thus, our findings suggest that MeCbl administration may improve the properties of the damaged skeletal muscle or degenerating muscle after denervation.

Abbreviations: MeCbl, Methylcobalamin; GM, Growth medium; DMEM, Dulbecco's modified Eagle's medium; BrdU, 5-Bromo-2-deoxyuridine; DM, Differentiation medium; PI3K, Phosphoinositide 3-kinase.

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2. Materials and Methods

2.1. Cell culture

C2C12 myoblasts were cultured under 5% CO_2 at 37 °C in growth medium (GM) consisting of Dulbecco's modified Eagle's medium (DMEM; GIBCO/BRL Life Technologies, Grand Island, NY; not including vitamin B12) supplemented with 10% fetal bovine serum (Gibco/BRL) and 1% penicillin and streptomycin.

2.2. Chemicals and antibodies

MeCbl was purchased from Sigma–Aldrich (St. Louis, MO). An MEK inhibitor, U0126, was purchased from Calbiochem (La Jolla, CA). Anti-p44/42 MAPK (Erk1/2) rabbit monoclonal antibody (1:1000), antiphospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) rabbit monoclonal antibody (1:1000), anti-Akt rabbit monoclonal antibody (1:1000), anti-phospho-Akt (Ser473) rabbit monoclonal antibody (1:1000), and anti-β-actin rabbit monoclonal antibody (1:1000) were purchased from Cell Signaling Technology (Beverly, MA). Horseradish peroxidase-conjugated anti-rabbit IgG antibody and ECL reagents were purchased from GE Healthcare (Little Chalfont, UK).

2.3. Immunocytochemistry

C2C12 cells were fixed with 4% paraformaldehyde in PBS for 20 min. After blocking nonspecific binding sites with 5% bovine serum albumin and 0.1% Tween 20 in PBS for 1 h, they were stained with DAPI (Wako Pure Chemical Industries, Osaka, Japan).

2.4. Cell proliferation assay

C2C12 cells were plated at a density of 5×10^3 cells in 6-cm plates and maintained in GM for 24 h prior to stimulation with MeCbl (10–100 μM) or U0126 (10 μM). At 1, 2, 3, and 4 days after the stimulation, they were trypsinized and resuspended. Cell counting was performed in triplicate on separate 10-μl aliquots using a hemacytometer. The proliferative capacity of C2C12 cells was also determined by cell proliferation ELISA using the 5-Bromo-2-deoxyuridine (BrdU) colorimetric system (Roche, Mannheim, Germany) according to the manufacturer's protocol. C2C12 cells were plated at 200 cells/well in a 96-well plate and cultured overnight in GM, followed by stimulation with MeCbl (10–100 μM) or U0126 (10 μM) for 72 h. The cells were labeled with BrdU (10 μM) for 2 h and reacted with anti-BrdU fluorescence-labeled antibody. Absorbance was directly measured using a spectrophotometric microplate reader (Thermo Fisher Scientific, Waltham, MA) at a test wavelength of 450 nm and a reference wavelength of 490 nm. This provided a measure of the degree of cell proliferation, and we termed it the proliferation index.

2.5. Western blotting

C2C12 cells were treated in the presence of MeCbl (10–100 μM) for 5 min with or without 10 μM U0126. They were homogenized with 100 μl of Kaplan buffer [150 mM NaCl, 50 mM Tris–HCl (pH 7.4), 1% NP-40, 10% glycerol, and a protease inhibitor cocktail] and clarified by centrifugation. Each sample including 18 μg of proteins was separated by SDS–PAGE and transferred onto polyvinylidene difluoride membranes. After blocking nonspecific binding sites with a blocking buffer [5% skimmed milk/1% Tween 20 in 20 mM TBS (pH 7.6)] for 1 h, the membranes were incubated overnight at 4 °C with monoclonal primary antibodies. Next, they were incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibody (1:1000) and subjected to ECL reagents. Protein expression levels were determined using the FAS-1000 system (Fuji Photo Film, Tokyo, Japan). Integrated optical densities of immunoreactive protein bands were measured using Scion Image software (Scion Corporation, Frederick, MD). To calculate normalized density, the density of phospho-Erk1/2 or phospho-Akt was divided by that of total Erk1/2 or total Akt, respectively, in the same membrane.

2.6. Wound healing assay

An *in vitro* wound healing assay was performed by the mechanical disruption of confluent monolayers of C2C12 cells as described previously [12]. C2C12 cells were cultured in 6-well plate in GM until they reached confluence. The cell layer was scratched using a scraper to form a continuous, well-delineated area of cellular disruption (4 mm). The cells were then rinsed twice with PBS to remove cellular debris and cultured in GM containing U0126 (10 μM). They were treated with MeCbl (10–100 μM) 1 h after U0126 administration. The medium was changed every other day. Using NIS Elements software (Nikon Instruments Inc, Melville, NY), the distance of the wound at an identical region of the plate was measured. The migration ratio was calculated by dividing the distance of the wound by the initial distance of the wound.

2.7. Apoptosis assay

To induce C2C12 cell differentiation, the cells were grown to approximately 90–100% confluence in GM and the medium was then switched to differentiation medium (DM) consisting of DMEM supplemented with 2% horse serum (Gibco/BRL) in the presence of MeCbl (10–100 μM) with or without U0126 (10 μM) for 3 days. Apoptosis was assessed using the cell death detection ELISA kit (Roche Diagnosis, Mannheim, Germany) according to the manufacturer's instructions. The degree of apoptosis was numerically evaluated by measuring the absorbance. The absorbance of each sample was normalized to that of the control group and designated the normalized absorbance ratio.

2.8. Statistical analysis

Data are expressed as the mean ± SEM. Statistical evaluation was performed by one-way ANOVA and *post hoc* Student's *t*-test.

3. Results

3.1. MeCbl promotes C2C12 cell proliferation via the Erk1/2 signaling pathway

In cases of muscle injury, the subsequent activation and proliferation of myogenic satellite cells are prerequisites for the regeneration of damaged muscle [7]. We focused on the effect of MeCbl on C2C12 cell proliferation. C2C12 cells were cultured with or without MeCbl for 4 days, and the total cell number was then counted every 24 h. Addition of MeCbl at a concentration of 100 μM increased the cell number to 1.29-fold compared with that of the control group ($p < 0.05$, Fig. 1A–D and I). C2C12 cell proliferation was also estimated by the method of BrdU uptake. The proliferation index increased on addition of MeCbl, even at the low concentration of 10 μM ($p < 0.05$, Fig. 1J).

Several intracellular signaling pathways are involved in the proliferation and differentiation of skeletal muscle cells. The activation of the Erk1/2 signaling pathway is known to be associated with myoblast proliferation [11,13], whereas its differentiation is promoted by the activation of the phosphoinositide 3-kinase (PI3K)/

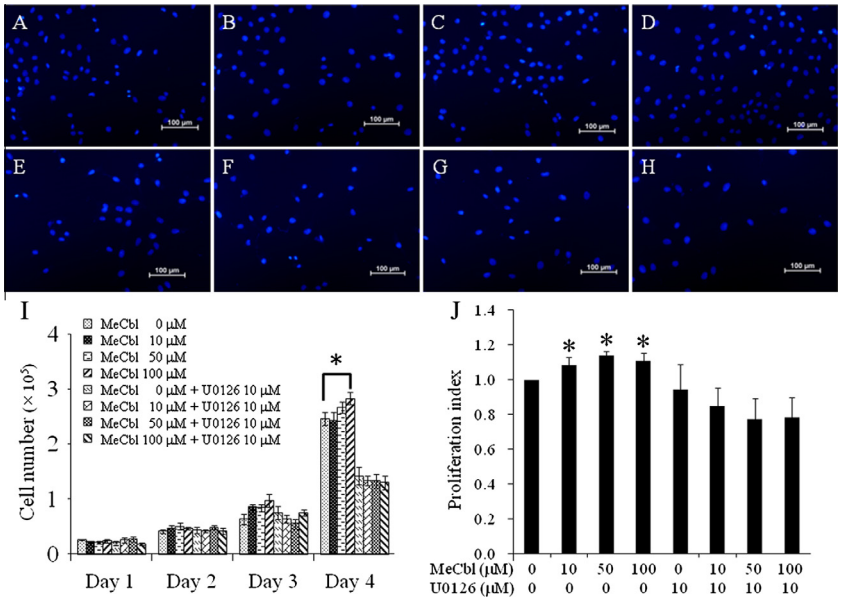

Fig. 1. MeCbl promotes C2C12 cell proliferation via the Erk1/2 signaling pathway. (A–H) Images showing nuclei (DAPI; blue) without MeCbl (A, E) and with MeCbl at 10 μM (B, F), 50 μM (C, G), and 100 μM (D, H). The cells in A–D were treated only with DMSO, and those in E–H were treated with 10 μM U1026. Bars indicate 100 μm. (I) Cell numbers for 4 days after treatment with or without MeCbl (10–100 μM) or U0126 (10 μM). Data are expressed as the mean ± SEM from 3 independent experiments (*$p < 0.05$). (J) Cell proliferation ELISA showing an MeCbl concentration-dependent increase in the absorbance of immunocytochemical staining of C2C12 cells with the proliferation marker BrdU for 72 h. Error bars indicate mean ± SEM. Results are representative of 8 independent experiments (*$p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Akt pathway [14,15]. To determine whether the signaling pathway plays a role in C2C12 cell proliferation by MeCbl, we estimated the activity of Erk1/2 and Akt by Western blotting. The activity of Erk1/2 increased to 1.49- and 1.44-fold compared with that of the control group 5 min after the addition of MeCbl at the concentration of 50 ($p < 0.05$) and 100 μM ($p < 0.01$), respectively (Fig. 2A). MeCbl did not affect the activity of Akt in C2C12 cells in GM (Fig. 2B). To estimate the duration of upregulated Erk1/2 activity by MeCbl, we observed the Erk activity for 72 h. The activation of the Erk1/2 was not observed 1, 6, 24, 48, and 72 h after the addition of MeCbl (Fig. 2C), whereas MeCbl increased its activity 5 min after the addition (Fig. 2A).

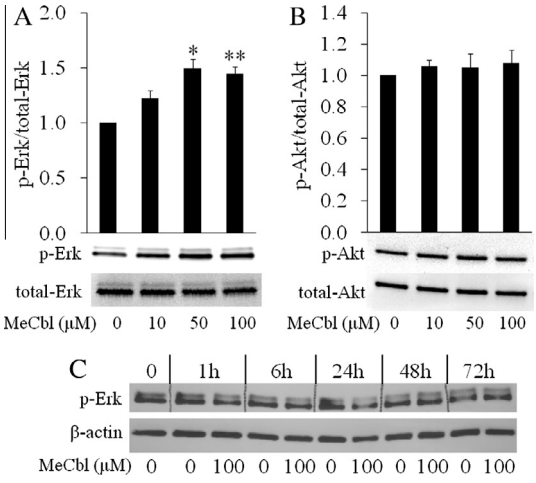

Fig. 2. MeCbl induces Erk1/2 phosphorylation in C2C12 cells C2C12 cells were stimulated with MeCbl for 5 min. Erk1/2 (A) and Akt (B) activities were detected by Western blotting. Quantification of normalized densities for Erk1/2 and Akt activities is shown. MeCbl increased Erk1/2 activity but did not affect Akt activity. Data are expressed as the mean ± SEM from 3 independent experiments (*$p < 0.05$, **$p < 0.01$). (C) The Erk1/2 activity was observed for 72 h after the stimulation with MeCbl. No difference in its activity was observed from 1 to 72 h.

Because the Erk1/2 signaling pathway and not Akt was activated by MeCbl in C2C12 cells in GM, U0126 (a specific Mek/Erk inhibitor) was also added to the proliferation assay in the presence of MeCbl. U0126 abolished C2C12 cell proliferation by MeCbl in terms of the total cell number (Fig. 1E–I) and BrdU uptake (Fig. 1J). These findings suggest that MeCbl accelerates C2C12 cell proliferation via the Erk1/2 signaling pathway.

3.2. C2C12 cell migration is promoted by MeCbl with Erk1/2 activity

The activation of the Erk1/2 signaling pathway is associated with not only proliferation but also migration of C2C12 cells [16]. Thus, we examined the effect of MeCbl on C2C12 cell migration in a wound healing assay. A layer of confluent C2C12 cells was scratched using a cell scraper, and the distance between the cells was measured. The initial distance of the wound did not differ significantly among all the groups (data not shown). On day 4, not day 3, we observed significant differences in the migration ratios between the control and MeCbl groups ($p < 0.05$, Fig. 3A–D and I). The Erk1/2 activity was increased by the addition of MeCbl especially on day 3 (Fig. 3J). The upregulated activity of the Erk1/2 on day 3 might lead to the promotion of the migration ratio on day 4. U0126 inhibited the promotion of the migration ratio on day 4 induced by MeCbl (Fig. 3E–H and I) with the deactivation of the Erk1/2 signaling pathway on day 3 and 4 (Fig. 3J). These results suggest that MeCbl promotes C2C12 cell migration via the Erk1/2 signaling pathway in manner similar to that of proliferation.

3.3. MeCbl inhibits apoptosis during C2C12 cell differentiation via the Erk1/2 signaling pathway

During differentiation *in vitro*, some myoblasts undergo apoptosis, while others withdraw from the cell cycle and form myotubes [17]. We also obtained the higher rate of apoptosis in C2C12 cells cultured in DM than in GM ($p < 0.05$, Fig. 4A). To analyze the role of MeCbl in apoptosis during cell differentiation, C2C12 cells cultured in DM were treated with MeCbl at concentrations of 10–

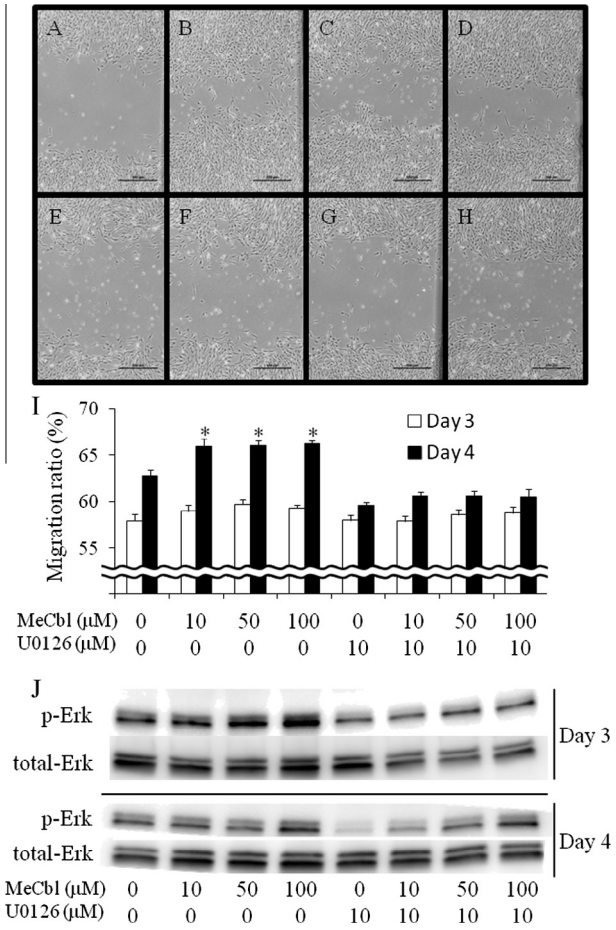

Fig. 3. MeCbl promotes C2C12 cell migration via the Erk1/2 signaling pathway. (A–H) Images of C2C12 cell migration at 4 days after scratching (×200) without MeCbl (A, E) and with MeCbl at 10 μM (B, F), 50 μM (C, G), and 100 μM (D, H). The cells in A–D were treated only with DMSO, and those in E–H were treated with 10 μM U1026. Bars indicate 500 μM. (I) The migration ratio after scratching was quantified on days 3 (white bars) and 4 (black bars). U0126 was added at a concentration of 10 μM. Error bars indicate mean ± SEM. Results are representative of 3 independent experiments (*$p < 0.05$ vs. control). (J) Erk1/2 activity at 3 and 4 days after scratching was detected by Western blotting with or without MeCbl (10–100 μM) or U0126 (10 μM).

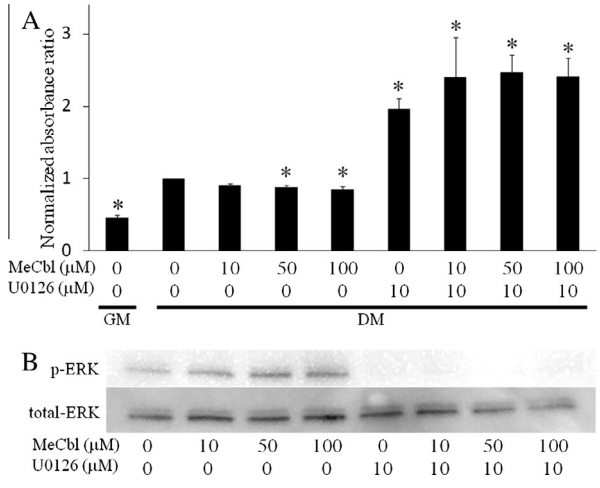

Fig. 4. MeCbl promotes the Erk1/2 activity and inhibits apoptosis during differentiation in C2C12 cell. (A) C2C12 cells were cultured in GM to the almost confluent condition. The medium was then switched to DM with or without MeCbl (10–100 μM) or U0126 (10 μM), and cells were maintained for 3 days. Error bars indicate mean ± SEM. Results are representative of 3 independent experiments (*$p < 0.05$ vs. control in DM). (B) Erk1/2 activity at 3 days after differentiation was detected by Western blotting with or without MeCbl (10–100 μM) or U0126 (10 μM).

100 μM and apoptotic cells were detected. This experiment revealed that the absorbance ratio for apoptotic cells was 12.2% and 14.4% lower in the MeCbl groups at concentrations of 50 and 100 μM, respectively, than that in the control group ($p < 0.05$), although there was no effect in the 10 μM MeCbl group (Fig. 4A). In C2C12 cells, the Erk1/2 signaling pathway plays important roles in not only proliferation and migration but apoptosis [18]. Thus the activation of Erk1/2 signaling pathway was observed during differentiation. MeCbl dose-dependently promoted the activation of Erk1/2 (Fig. 4B). The addition of U0126 increased the apoptotic ratio in the presence or absence of MeCbl (Fig. 4A) with the inactivation of the Erk1/2 (Fig. 4B). These findings suggest that MeCbl inhibits apoptosis during C2C12 cell differentiation with the activation of the Erk1/2 signaling pathway.

4. Discussion

The motor unit in the peripheral nervous system comprises a motoneuron axon terminal, terminal Schwann cell, and muscle. After a motoneuron axon is injured, Wallerian degeneration occurs and the distal part of the injury site degenerates. This mechanism is a prerequisite for the regeneration of motoneuron axons; however, long-term denervation contributes to irreversible changes in the muscle, such as fiber atrophy and fibrosis. In this situation, complete recovery or regeneration of a motor unit cannot be achieved even if the injured axons recover completely, and this results in a serious clinical problem. Preventing degeneration or fibrosis of denervated muscle fibers is very important for regenerating a motor unit in the same manner as axonal regeneration. In this study, we focused on the effects of MeCbl, an analog of vitamin B12, on muscle and found that MeCbl could promote the proliferation and migration of C2C12 cells via the Erk1/2 signaling pathway.

We demonstrated that MeCbl promoted Erk1/2 activation in C2C12 cells (Figs. 2A and 3J). The Erk1/2 signaling pathway is activated in the skeletal muscle in response to physical exercise or injury and plays an important role during myogenesis. Several studies have found that the Erk1/2 signaling pathway promotes proliferation and migration [11,19,20], and our experiments demonstrated that MeCbl promoted the proliferation (Fig. 1) and migration (Fig. 3) of C2C12 cells via the Erk1/2 signaling pathway. However, we found the discrepancies in the duration of the Erk1/2 activation by MeCbl between the cell proliferation assay and the wound healing assay. The activation of Erk1/2 was observed only at the time point of 5 min after the addition of MeCbl, not at 1, 6, 24, 48, 72 h in the cell proliferation assay (Fig. 2A and C), whereas its activity by MeCbl was maintained for several days in the wound healing assay (Fig. 3J). In an *in vitro* wound healing assay using C2C12 cells, phosphorylated Erk1/2 levels reportedly peaked at 10 min after wounding and reached baseline levels at 1 h after wounding [12]. In our experiment, addition of MeCbl maintained phosphorylated Erk1/2 at levels higher than that of the control on days 3 and 4 (Fig. 3J); therefore, the promotion of Erk1/2 activity by MeCbl was not influenced by the phosphorylation of Erk1/2 by mechanical stress induced by scratching monolayer cells in our wound healing assay. We previously reported that MeCbl promoted axonal outgrowth with increased Erk1/2 activity in cerebellar granule neurons and dorsal root ganglion neurons [2]. MeCbl activated Erk1/2 in neurons for 72 h, whereas brain-derived neurotrophic factor activated it for 1 h, suggesting that the mechanisms for activating Erk1/2 differed between MeCbl and neurotrophin like brain-derived neurotrophic factor. The detailed mechanism is unknown, but the activation of Erk1/2 by MeCbl for several days in neurons and in the wound healing assay of C2C12 cells may be maintained by the methylation of some protein kinases [21]. MeCbl may possibly prevent the muscle degeneration or fibrosis

resulting from muscle injury or denervation by promoting axonal outgrowth in neurons and the proliferation and migration of myoblasts accompanied by Erk1/2 activity for long periods.

The PI3K/Akt signaling pathway plays a central role in inhibiting apoptosis [22,23]. In C2C12 cells, Akt plays a protective role in thimerosal-induced apoptosis in GM [24]. Other reports indicated that 17β-estradiol exerts anti-apoptotic effects through the PI3K/Akt signaling pathway in C2C12 cells exposed to hydrogen peroxide [25] and under these conditions Erk2 also plays an important role in the upstream signaling of Akt [18]. Thus, both the Erk and Akt signaling pathways may play important roles in the anti-apoptotic effects in C2C12 cells. We focused on the Erk1/2 signaling pathway in apoptosis during differentiation. The Erk1/2 signaling pathway has reported to be activated in undifferentiated myoblasts and postmitotic myotubes and to be downregulated during the early differentiation stage and its activation is required for optimal differentiation and myotube survival [11]. These facts may support our data that MeCbl promoted the Erk1/2 activation (Fig. 4B) and inhibited the apoptotic rate (Fig. 4A) 3 days after differentiation. Moreover, inactivation of the Erk1/2 pathway by U0126 accelerated the apoptotic rate (Fig. 4A and B). The biphasic activation of the Erk1/2 signaling pathway plays an important role in the proliferation and late stage differentiation in C2C12 cells and MeCbl might have the ability to support its process.

In conclusion, our results indicate that MeCbl promotes the proliferation and migration of C2C12 cells cultured in GM and inhibits apoptosis during differentiation process via the Erk1/2 signaling pathway. We propose that MeCbl may improve muscle injury or prevent muscle degeneration after denervation.

References

[1] G. Scalabrino, B. Monzio-Compagnoni, M.E. Ferioli, E.C. Lorenzini, E. Chiodini, R. Candiani, Subacute combined degeneration and induction of ornithine decarboxylase in spinal cords of totally gastrectomized rats, Lab. Invest 62 (1990) 297–304.

[2] K. Okada, H. Tanaka, K. Temporin, M. Okamoto, Y. Kuroda, H. Moritomo, T. Murase, H. Yoshikawa, Methylcobalamin increases Erk1/2 and Akt activities through the methylation cycle and promotes nerve regeneration in a rat sciatic nerve injury model, Exp. Neurol. 222 (2010) 191–203.

[3] K. Okada, H. Tanaka, K. Temporin, M. Okamoto, Y. Kuroda, H. Moritomo, T. Murase, H. Yoshikawa, Akt/mammalian target of rapamycin signaling pathway regulates neurite outgrowth in cerebellar granule neurons stimulated by methylcobalamin, Neurosci. Lett. 495 (2011) 201–204.

[4] K. Yamazaki, K. Oda, C. Endo, T. Kikuchi, T. Wakabayashi, Methylcobalamin (methyl-B12) promotes regeneration of motor nerve terminals degenerating in anterior gracile muscle of gracile axonal dystrophy (GAD) mutant mouse, Neurosci. Lett. 170 (1994) 195–197.

[5] M. Sonobe, H. Yasuda, I. Hatanaka, M. Terada, M. Yamashita, R. Kikkawa, Y. Shigeta, Methylcobalamin improves nerve conduction in streptozotocin-diabetic rats without affecting sorbitol and myo-inositol contents of sciatic nerve, Horm. Metab. Res. 20 (1988) 717–718.

[6] T. Watanabe, R. Kaji, N. Oka, W. Bara, J. Kimura, Ultra-high dose methylcobalamin promotes nerve regeneration in experimental acrylamide neuropathy, J. Neurol. Sci. 122 (1994) 140–143.

[7] T.J. Hawke, D.J. Garry, Myogenic satellite cells: physiology to molecular biology, J. Appl. Physiol. 91 (2001) 534–551.

[8] Y. Iwata, N. Ozaki, H. Hirata, Y. Sugiura, E. Horii, E. Nakao, M. Tatebe, N. Yazaki, T. Hattori, M. Majima, N. Ishiguro, Fibroblast growth factor-2 enhances functional recovery of reinnervated muscle, Muscle Nerve 34 (2006) 623–630.

[9] C. Lazerges, P.A. Daussin, B. Coulet, R. Boubaker el Andalousi, J.P. Micallef, M. Chammas, Y. Reyne, F. Bacou, Transplantation of primary satellite cells improves properties of reinnervated skeletal muscles, Muscle Nerve 29 (2004) 218–226.

[10] N.C. Jones, Y.V. Fedorov, R.S. Rosenthal, B.B. Olwin, ERK1/2 is required for myoblast proliferation but is dispensable for muscle gene expression and cell fusion, J. Cell Physiol. 186 (2001) 104–115.

[11] Z. Wu, P.J. Woodring, K.S. Bhakta, K. Tamura, F. Wen, J.R. Feramisco, M. Karin, J.Y. Wang, P.L. Puri, P38 and extracellular signal-regulated kinases regulate the myogenic program at multiple steps, Mol. Cell Biol. 20 (2000) 3951–3964.

[12] K. Yeow, C. Cabane, L. Turchi, G. Ponzio, B. Derijard, Increased MAPK signaling during in vitro muscle wounding, Biochem. Biophys. Res. Commun. 293 (2002) 112–119.

[13] D.J. Milasincic, M.R. Calera, S.R. Farmer, P.F. Pilch, Stimulation of C2C12 myoblast growth by basic fibroblast growth factor and insulin-like growth factor 1 can occur via mitogen-activated protein kinase-dependent and -independent pathways, Mol. Cell Biol. 16 (1996) 5964–5973.

[14] Y. Tamir, E. Bengal, Phosphoinositide 3-kinase induces the transcriptional activity of MEF2 proteins during muscle differentiation, J. Biol. Chem. 275 (2000) 34424–34432.

[15] Q. Xu, Z. Wu, The insulin-like growth factor-phosphatidylinositol 3-kinase-Akt signaling pathway regulates myogenin expression in normal myogenic cells but not in rhabdomyosarcoma-derived RD cells, J. Biol. Chem. 275 (2000) 36750–36757.

[16] V. Odemis, K. Boosmann, M.T. Dieterlen, J. Engele, The chemokine SDF1 controls multiple steps of myogenesis through atypical PKCzeta, J. Cell Sci. 120 (2007) 4050–4059.

[17] J. Wang, K. Walsh, Resistance to apoptosis conferred by Cdk inhibitors during myocyte differentiation, Science 273 (1996) 359–361.

[18] A.C. Ronda, A. Vasconsuelo, R. Boland, Extracellular-regulated kinase and p38 mitogen-activated protein kinases are involved in the antiapoptotic action of 17beta-estradiol in skeletal muscle cells, J. Endocrinol. 206 (2010) 235–246.

[19] S.A. Coolican, D.S. Samuel, D.Z. Ewton, F.J. McWade, J.R. Florini, The mitogenic and myogenic actions of insulin-like growth factors utilize distinct signaling pathways, J. Biol. Chem. 272 (1997) 6653–6662.

[20] L. Yahiaoui, D. Gvozdic, G. Danialou, M. Mack, B.J. Petrof, CC family chemokines directly regulate myoblast responses to skeletal muscle injury, J. Physiol. 586 (2008) 3991–4004.

[21] J.I. Toohey, Vitamin B12 and methionine synthesis: a critical review. Is nature's most beautiful cofactor misunderstood?, BioFactors 26 (2006) 45–57

[22] S.G. Kennedy, A.J. Wagner, S.D. Conzen, J. Jordan, A. Bellacosa, P.N. Tsichlis, N. Hay, The PI 3-kinase/Akt signaling pathway delivers an anti-apoptotic signal, Genes Dev. 11 (1997) 701–713.

[23] T.F. Franke, C.P. Hornik, L. Segev, G.A. Shostak, C. Sugimoto, PI3K/Akt and apoptosis: size matters, Oncogene 22 (2003) 8983–8998.

[24] W.X. Li, S.F. Chen, L.P. Chen, G.Y. Yang, J.T. Li, H.Z. Liu, W. Zhu, Thimerosal-induced apoptosis in mouse C2C12 myoblast cells occurs through suppression of the PI3K/Akt/survivin pathway, PLoS One 7 (2012) e49064.

[25] A. Vasconsuelo, L. Milanesi, R. Boland, 17Beta-estradiol abrogates apoptosis in murine skeletal muscle cells through estrogen receptors: role of the phosphatidylinositol 3-kinase/Akt pathway, J. Endocrinol. 196 (2008) 385–397.