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Enigma homolog 1 promotes myogenic gene expression and differentiation of C2C12 cells

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ABSTRACT

The Enigma homolog (ENH) gene generates several splicing variants. The initially identified ENH1 possesses one PDZ and three LIM domains, whereas ENH2∼4 lack the latter domains. The splicing switch from ENH1 to LIM-less ENHs occurs during development/maturation of skeletal and heart muscles. We examined for the roles of ENH splicing variants in muscle differentiation using C2C12 cells. Cells stably expressing ENH1 exhibited significantly higher MyoD and myogenin mRNA levels before differentiation and after 5 days in low serum-differentiating medium than mock-transfected cells. ENH1-stable transformants also retained the ability to exhibit elongated morphology with well-extended actin fibers following differentiation. In contrast, cells stably expressing ENH3 or ENH4 did not show myotube-like morphology or reorganization of actin fibers following culture in the differentiating medium. Transient overexpression of ENH1 using adenovirus supported the increased expression of muscle marker mRNAs and the formation of well-organized stress fibers, whereas ENH4 overexpression prevented these morphological changes. Furthermore, specific suppression of ENH1 expression by RNAi caused a significant reduction in MyoD mRNA level and blocked the morphological changes. These results suggest that ENH1 with multiple protein–protein interaction modules is essential for differentiation of striated muscles, whereas ectopic expression of LIM-less ENH disrupts normal muscle differentiation.

1. Introduction

PDZ–LIM proteins play pivotal roles in differentiation and function of various cell types. The primary action of these proteins is thought to arise from their ability to interact with multiple structural and functional molecules. PDZ domains bind to cytoskeletal and membrane proteins, whereas LIM domains interact with various signaling molecules including protein kinases and transcription factors. Therefore, PDZ–LIM proteins act as scaffolds to localize and assemble various functional molecules at cytoskeletal structures and underneath the plasma membrane for effective sorting and coupling of intracellular signaling events. Several PDZ–LIM proteins are highly expressed and play important roles in striated muscles. For example, PDZ domains of CLP36 (PDLIM1), ALP (PDLIM3) and Cypher (PDLIM6) tether their associated proteins at the Z-disk by binding to α-actinin [1–3]. LIM domains of these proteins appear to interact with protein kinase Cs. These localized assemblies are essential for the formation of proper muscle structures [4,5]. Since Ca^{2+} concentrations are much higher near the Z-disk, the PDZ–LIM protein-based scaffolding of these kinases may confer their localized activation, and as a result, the proper organization and functioning of striated muscles.

Enigma homolog 1 (ENH1/PDLIM5) is another member of PDZ–LIM proteins that is highly expressed in skeletal and heart muscles. ENH1 possesses a PDZ domain in the N-terminal region and three LIM domains in the C-terminal portion [6]. Similarly to other PDZ–LIM members in striated muscles, the PDZ domain of ENH1 binds to α-actinin, whereas its LIM domains bind to several protein kinase Cs [6,7] and protein kinase D [8]. In addition, we identified that ENH1 simultaneously binds to protein kinase D and L-type calcium channel in cardiac tissue [8]. This ENH1-based ternary complex is required for effective protein kinase D-dependent inhibition of the channel activity. Finally, the study in neurocarcinoma revealed that the transcription regulator Id2 binds to the ENH1 LIM domains [9]. The high level of ENH1 in these cancer cells is proposed to trap this factor in cytosol, preventing neuronal differentiation and promoting cell proliferation. Thus, the abilities of ENH1 to localize at certain subcellular sites and to interact with multiple functional proteins are important for its biological functions.

Although it is clear that ENH1 acts as an anchoring protein, the gene for ENH1 generates several splicing variants (ENH2∼4) that lack all three LIM domains. In particular, LIM-less ENHs are predominant in striated muscles of adult animals [10]. Our recent

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analysis revealed the splicing switch from three LIM-containing ENH1 to LIM-less ENHs during postnatal heart development and differentiation of C2C12 cells [11]. Therefore, LIM-less ENHs must possess important, yet unknown, functions in mature striated muscles. These findings also suggest that distinct ENH splicing variants play different roles in developing and mature striated muscles. To address this issue, we altered the levels of ENH splicing variants in C2C12 cells to test for their effects on myogenic differentiation. ENH1 appeared to promote expression of myogenic genes and myotube formation, whereas LIM-less ENH4 disrupts myotube-like morphological changes.

2. Materials and methods

2.1. Cell culture

Mouse skeletal C2C12 cells were obtained from RIKEN BRC Cell Bank (Ibaraki, Japan). Cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum (Gibco, Auckland, NZ), 50 U/ml penicillin and 50 μg/ml streptomycin (Nacalai Tesque, Kyoto, Japan) under 5% CO_2 atmosphere at 37 °C. For differentiation, cells were passed on plastic or glass-bottom dishes at ~50% and cultured until semi-confluence (~90%). Culture medium was then switched to 2% fetal bovine serum-containing medium. The medium was changed once every three days during the entire experimental period.

2.2. Plasmid constructions and isolation of stable ENH-expressing cells

To establish stable ENH variant-expressing cells, we constructed cDNAs for ENH variants with 3xFlag tag at the N-terminus (p3xFLAG-CMV-10 vector, Sigma–Aldrich). Briefly, ENH1, ENH3 and ENH4 cDNAs were amplified from the previously obtained rat clones [12] as a template by PCR using primers with a restriction enzyme site: 5′-GACAAGCTTATGAGCAACTACAATGTGTCA TTGG-3′, and 5′-GAGCGGATATCTCAGAAATTCACAGAATGAGCATG-3′ for ENH1 or 5′-GAGATGATATCTCACTGTACATTAAGAGCACG-3′ for ENH3 and ENH4 (the underlined sequences indicate *Hind*III and *Eco*RV sites). Following digestion with *Hind*III and *Eco*RV, the digested PCR fragments were cloned into the corresponding sites of the p3xFLAG-CMV-10 vector.

C2C12 cells were transfected with 2 μg of the generated constructs or pcDNA3 vector as a mock control using the lipofectamine 2000 Reagent (Invitrogen). Stable transformants were obtained and maintained with the standard culture medium supplemented with 800 μg/ml of G418 (Nacalai Tesque, Kyoto, Japan).

2.3. Western Blotting

Lysates were prepared from cultured cells using Lysis buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 1 mM Na_3VO_4, 1% Triton-X100, and protease inhibitors (Roche)). Proteins were separated on a 10% SDS–polyacrylamide gel and transferred to polyvinylidene difluoride membrane (Millipore). The membrane was coated with 5% nonfat dry milk, probed with anti-FLAG M2 monoclonal antibody (Sigma–Aldrich), and then with anti-mouse donkey IgG linked to horseradish peroxidase (Jackson Immuno Research). Immunoreactive proteins were detected using chemiluminescent reagents (Thermo-Fisher).

2.4. Adenovirus vector constructions and infection

The 'hairpin strategy' was used to design small interfering RNA towards ENH1. We chose the target sequence from the coding region unique in both mouse and rat ENH1, but not present in other splicing variants in either species, corresponding to the positions 1698–1718 (GenBank Accession # NM_053326.1). A synthetic oligonucleotide containing the target hairpin sequence inserted with a 9-bp loop and a 6-bp adenosine tandem at the 5′end was cloned behind the U6 promoter of the vector, as described previously [8]. Adenoviruses carrying full-length ENH1 or ENH4 cDNA, as well as control GFP-expressing viruses, were prepared as described previously [12]. We infected C2C12 cells two day after switching to low serum-containing differentiation medium. Infection efficiency was ~90% judged by the ratio of GFP- or anti-Flag antibody-positive cells to total cells one day after infection.

2.5. Real-time PCR analysis

Real-time PCR analysis was used to determine steady state mRNA levels of ENH splicing variants and myogenic marker genes. Individual ENH variant mRNAs were amplified using previously described primers which reacted with both endogenous mouse and exogenous rat cDNAs [11], whereas skeletal marker genes were detected with primers as follows: mouse myogenic differentiation 1 (MyoD) (GenBank Accession #: NM_010866.2), 5′-CTGCTCCTTTGAGACAGCAGAC-3′(Nucleotide 259–280) and 5′-TTG CGCTTGCACGCCTTGCA-3′(Nucleotide 491–510); and myogenin (GenBank Accession #: NM_031189), 5′-TCACATAAGGCTAACACC-CAGC-3′ (Nucleotide 876–896) and 5′-GCACTCATGTCTCT-CAAACGGT-3′ (Nucleotide 1090–1069). Synthesis of cDNA and PCRs were performed as described previously [11].

The mRNA levels were normalized using cyclophilin mRNA as a control. Statistical analysis was performed by one-way *ANOVA*, followed the layered *Bonferroni's* test. Data are presented as the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.0001$ compared to control samples.

2.6. Fluorescent immunostaining

Cells in a collagen-coated 35-mm^2 glass-bottom dish (Matsunami Glass Ind.) were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. Cells were probed with anti-FLAG M2 monoclonal antibody (Sigma–Aldrich). Immunostaining was revealed with Alexa Fluor 488-conjugated secondary antibody (Invitrogen). Staining of F-Actin was performed using rhodamine-conjugated phalloidin (Cytoskeletons). Nuclei were visualized with 4′,6-diamidino-2-phenylindole (DAPI, Dojindo Molecular Technologies). Images were captured and processed with Fluoview FV1000 confocal laser scanning microscope (Olympus).

3. Results

3.1. Myogenic differentiation of C2C12 cells stably expressing ENH splicing variants

Expression of ENH variants changes during differentiation of C2C12 cells [11]. Three LIM-containing ENH1 declines during differentiation, while LIM-less ENH4 gradually increases during differentiation. Thus, these cells provide a useful system to test for the roles of distinct ENH splicing variants in myogenic differentiation. We first established C2C12 cells that stably expressed Flag-tagged ENH1, ENH3 or ENH4 (Fig. 1A). Stable transformants contained significant and similar amounts of anti-Flag antibody-reactive proteins with the expected molecular weights (Fig. 1B). In the standard growth medium, these transformants, as well as mock-transfected cells, showed no apparent morphological differences compared to untransfected original C2C12 cells (Fig. 1C). All ENH variants detected with anti-Flag antibody were localized at

the edges of cells with some stress fiber-like structures. This localization is consistent with the presence of PDZ domain in all variants which binds to cytoskeleton via α-actinin. Upon serum reduction, control mock cells changed morphology, forming elongated tube-like structures. ENH1-expressing cells also showed similar elongated morphology, though the change was less dramatic. Exogenously expressed ENH1 proteins were found in muscle fiber-like structures. In contrast, the switching to low serum-containing medium failed to produce myotube-like morphological changes in ENH3- or ENH4-expressing cells. ENH3 and ENH4 proteins in cells cultured in the differentiation medium were seen at the periphery of the nucleus in addition to cell edges and cytoskeletons, suggesting that some overexpressed LIM-less ENHs were localized in non-actin-based structures. Thus, stable expression of PDZ- and LIM-containing ENH1 undisturbed myotube-like morphological transformation, whereas LIM-less ENHs disrupts it during differentiation of C2C12 cells.

Differentiation of C2C12 cells is associated with ordered changes in the expression of various skeletal muscle marker genes. We measured mRNA levels for the two key skeletal muscle transcription factors, MyoD and myogenin, during differentiation of ENH variant-expressing cells. The basal myogenin mRNA level before differentiation was higher in the ENH1-expressing cells than mock cells (Fig. 1D, Day 0). Stable ENH1 expression also enhanced mRNA levels of the two transcription factors upon differentiation (Fig. 1D, Day 5). Consistent with its morphological effects, stable ENH4 expression reduced the induction of myogenin mRNA after culture in differentiation medium for 5 days. However, stable ENH3 expression resulted in increased expression of MyoD mRNA after culture in low serum medium. Likewise, stable ENH3 transformants, as well as ENH4 transformants, tended to have higher MyoD and myogenin mRNA levels following culture in low serum medium than mock cells. These results indicate that stable expression of ENH1 promotes the transcription of skeletal transcription factor genes. LIM-less ENH-stable transformants, however, appear to exhibit apparent mismatches between morphological changes and skeletal marker gene expression.

3.2. Differentiation of C2C12 cells transiently expressing ENH splicing variants or shRNA against ENH1

Selection of stable transformants is often associated with adaptive or compensatory changes. To further test for the stimulatory and inhibitory roles of ENH splicing variants, we used adenovirus-mediated transient overexpression of ENH1 and ENH4, as well as shRNA against ENH1. Altered exogenous and endogenous ENH variant mRNA levels were confirmed by real-time PCR analysis with primers that reacted with both exogenous rat and endogenous mouse transcripts (Fig. 2A). Selective changes in ENH splicing variant mRNAs were seen one day after infection (total three days in low serum medium, Day 3), whereas altered ENH variant mRNA

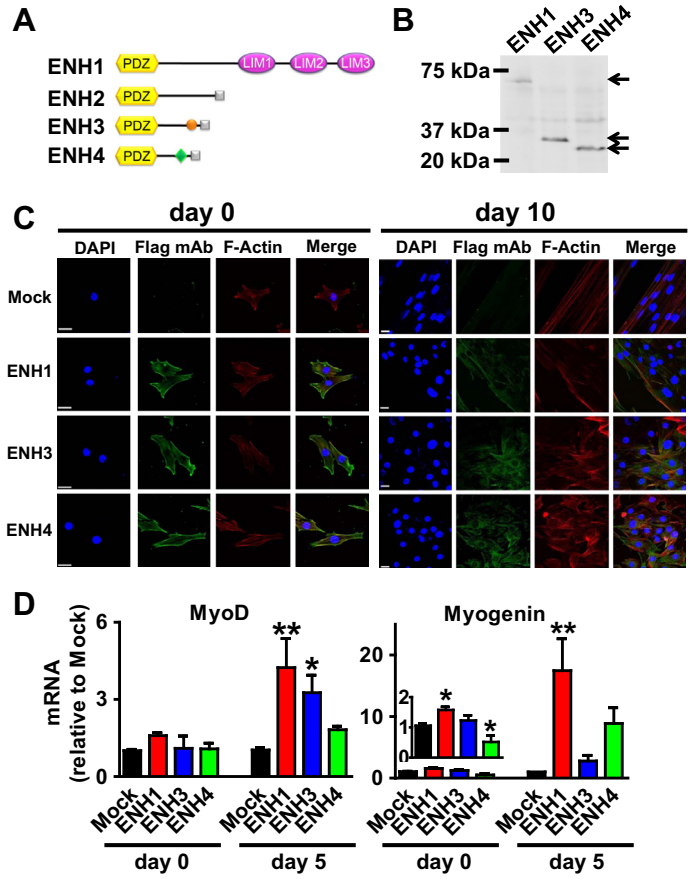

Fig. 1. Differentiation of C2C12 cells stably expressing individual ENH splice variants. (A) A diagram shows structural organizations of ENH splicing variants [10]. The square symbols represent a region common among all LIM-less ENHs. Round and pentagon symbols indicate portions unique in ENH3 and ENH4, respectively. (B) Western blot analysis indicates the expression of Flag-tagged ENH splice variants in stable transformants. Arrows indicate the positions of each ENH variants. (C) Pictures show confocal laser scanning images of stable transformants before (Day 0) and after 10 days in low-serum differentiation medium (Day 10). Cells were stained with DAPI (nuclei), anti-Flag antibody (Flag mAb), rhodamine-conjugated falloidin (F-actin), as described in Materials and Methods. Bars in the pictures indicate the scale of 20 μm. (D) Graphs show the mRNA levels for skeletal muscle specific transcription factor, MyoD and myogenin in the indicated transformants. The mRNA levels were determined by real-time PCR. Bars and error bars indicate the mean ± SEM ($n = 4$).

levels were returned to the levels in control GFP-expressing virus-infected cells five days after infection (Day 7).

As expected, tube-like morphological changes were seen in control GFP virus-infected cells following extended culture in low serum-containing medium. ENH1-overexpressing cells were also capable of forming elongated actin fibers. However, we observed little tube-like multinucleated cells typical in control or uninfected C2C12 cells. ENH4 overexpression, as well as suppression of endogenous ENH1 expression by shRNA prevented the formation of elongated morphology or actin fibers (Fig. 2B). We next determined mRNA levels for MyoD and myogenin in these virus-infected cells (Fig. 2C). After three days in differentiation medium, overexpression of ENH1 and ENH1 shRNA increased and decreased MyoD mRNA level, respectively. Similar tendencies were seen with myogenin mRNA level, though no significant changes were detected. Increased myogenin mRNA level became significant at 7 days in differentiation. Overexpression of ENH4 had no effects on MyoD or myogenin mRNA level at the early or late days in the low-serum medium. Taken together, LIM-containing ENH1 stimulates the expression of myogenic transcription factors and the formation of extended actin fibers, whereas LIM-less ENH4 disrupts morphological changes without alterations in the expression of the myogenic transcription factors.

4. Discussion

Deletion of the ENH gene appeared to cause disorganized Z-disk structures and contraction defects of the heart [13]. However, the presence of distinct ENH splicing variants makes it hard to identify

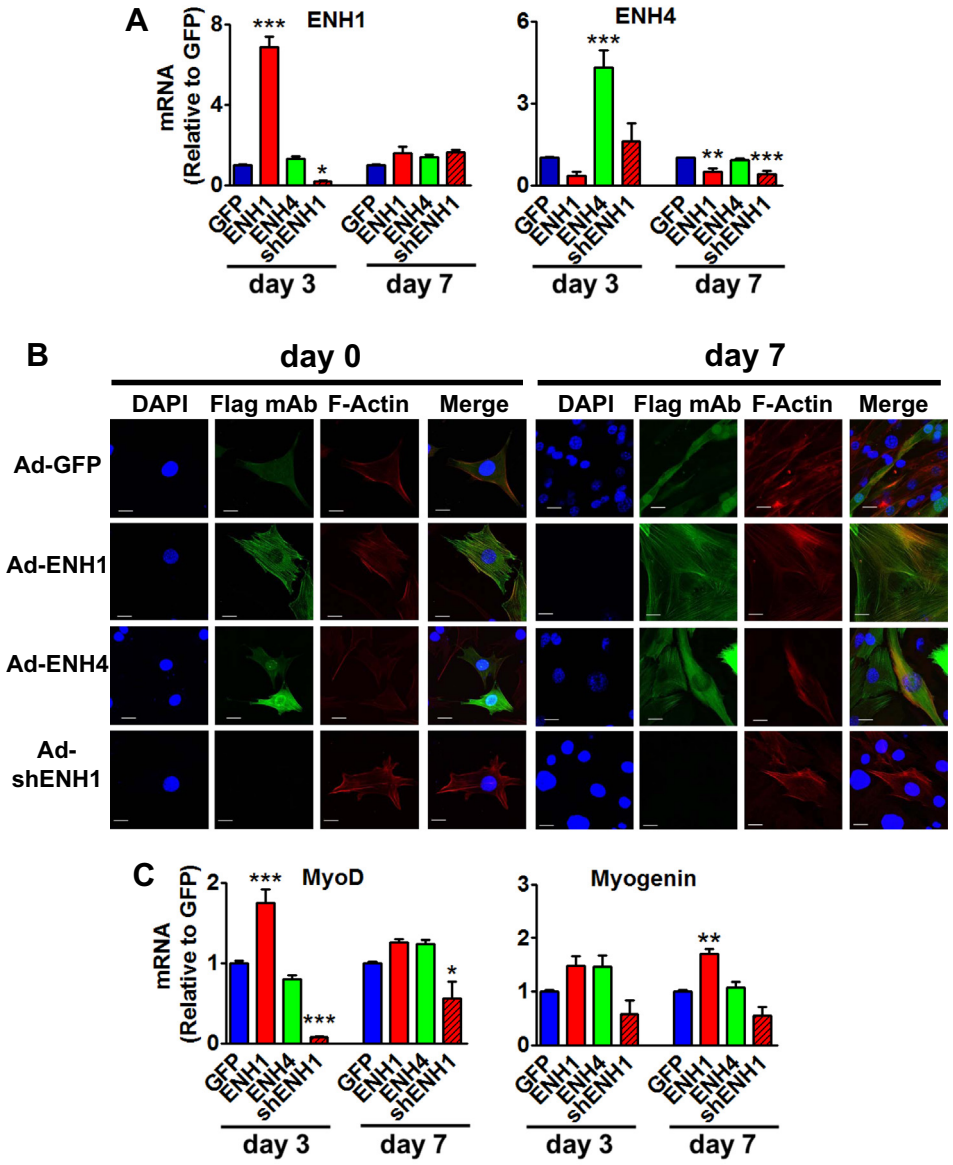

Fig. 2. Differentiation of C2C12 cells transiently expressing individual ENH splice variants or shRNA against ENH1. (A) Graphs show mRNA levels of ENH1 and ENH4. Real-time PCR were performed with primers that detected both endogenous and exogenous ENHs. ENH1 and 4 mRNA levels were normalized with cyclophilin mRNA. Relative to GFP. Bars and error bars indicate the mean + SEM of ENH mRNA levels relative to GFP mRNA ($n = 3$). (B) Pictures show confocal laser scanning images of cells infected with adenovirus carrying individual ENH variants or GFP. C2C12 cells were infected by adenovirus at 2 days after switching to the low serum-containing medium. Staining was performed as described in the legend for Fig. 1. Bars indicate the scale of 20 μm. (C) Graphs show the levels of MyoD and myogenin mRNAs. The mRNA levels were normalized by cyclophilin mRNA, and are presented as the values relative to GFP ($n = 3$).

specific molecular defects that lead to these morphological and functional abnormalities. ENH1 is abundant in less-differentiated immature striated muscles, whereas ENH3 and ENH4 are predominant forms in adult heart and skeletal muscle, respectively [11]. These splicing switches during development/maturation of striated muscles raised the possibility that distinct ENH splicing variants might play distinct biological functions in immature and mature muscle cells [11]. The present study tested this possibility using differentiation of C2C12 cells. Our results indicate that multiple domain-containing ENH1 is essential for the expression of myogenic genes and the formation of elongated actin fibers during C2C12 cell differentiation. Furthermore, ectopic expression of LIM-less ENH disrupts morphological changes. Thus, the abundant expression of ENH1, along with the lack of LIM-less ENHs, in immature striated muscles promotes their proper differentiation/maturation.

Cellular differentiation and morphological changes of skeletal muscle is controlled by a set of transcription factors called myogenic regulatory factors [14]. The myogenic factor 5 (Myf5) and muscle-specific regulatory factor 4 (Mrf4, also known as Myf6) define early skeletal muscle precursor cells. The myoblast determination protein (MyoD) and myogenin are then expressed in differentiating myofibers. C2C12 cells are Myf5-positive muscle precursor cells, often considered as muscle satellite cells present in mature muscle. Therefore, the key event in muscle differentiation of C2C12 cells and native Myf5-positive precursors is the expression of MyoD and its related factors, such as myogenin. In this study, we found that ENH1 overexpression led to upregulation of MyoD and myogenin, although the degrees of changes were variable depending on the experimental conditions. In addition, knocking down ENH1 expression significantly reduced MyoD expression. These data suggest that the primaryaction of ENH1 might be stimulation of the transcription of MyoD and/or myogenin genes. These myogenic transcription factors are bHLH proteins, which are known to increase own transcription [15]. Morover, ENH1 has been shown to trap the transcription repressor Id2 that forms complexes with bHLH proteins [9]. Thus, one possibility is that ENH1 traps Id2 or related transcription repressor to the cytosol, allowing positive autoregulation of MyoD and myogenin gene transcription to proceed.

The present study also suggests the inhibitory action of LIM-less ENH in myogenic differentiation. Our previous work identified similar agonistic and antagnonistic actions of ENH1 and LIM-less ENH in cardiomyocyte hypertrophy [12]. In cultured neonatal myocytes, ENH1 overexpression increased the expression of hypertrophy marker genes and the cell size, whereas ENH4 overexpression prevented these hypertrophic changes induced by stimulation with several hormones [12]. In both cases, the inhibitory actions of LIM-less ENH might arise from its forced replacement of ENH1 from the site of action. This dominant-negative action of PDZ-only ENH against ENH1 is easily imaginable; however, muscles appear to express many other PDZ–LIM proteins that bind to α-actinin. For example, CLP36 (PDLIM1), ALP (PDLIM3) and Cypher (PDLIM6) are α-actinin-binding PDZ domain-containing proteins abundant in skeletal and heart muscles [1–3]. Therefore, LIM-less ENH might act as a dominant-negative subunit against not only ENH1 but also other PDZ-LIM proteins.

It is also important to note that overexpression of LIM-less ENHs resulted in inconsistent changes in cell morphology and expression of myogenic transcription factors. Both stable and transient expression of LIM-less ENHs blocked the appearance of myotube-like morphology and elongated actin fibers during C2C12 cell differentiation. In contrast, their influences on expression of MyoD and myogenin genes appeared variable and in some cases inconsistent with their prevention of myogenic morphological changes. Although the reasons for these variable outcomes remain unclear, the observations suggest that LIM-less ENHs act at the downstream of these transcription factors to block the morphological changes. Given that the stimulation of differentiation by ENH1 is likely due to its activation of myogenic genes, the simple antagonism against this ENH1 action could not account for the inhibitory action of LIM-less ENHs. Rather, LIM-less ENHs may inhibit expression of downstream genes or structural organization of actin fibers.

Taken together, the present study shows that ENH1 with the two distinct interaction domains is essential for myogenic gene expression and morphological transformation during myogenic differentiation of C2C12 cells. On the contrary, PDZ-only ENHs exhibit the inhibitor action against these morphological changes with unknown mechanisms. Further understanding about physiological actions of LIM-less ENHs in mature striated muscles may provide the clues for the molecular mechanisms.

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References

[1] H. Wang, D.C. Harrison-Shostak, J.J. Lemasters, B. Herman, Cloning of a rat cDNA encoding a novel LIM domain protein with high homology to rat RIL, Genetics 165 (1995) 267–271.
[2] H. Xia, S.T. Winokur, W.L. Kuo, M.R. Altherr, D.S. Bredt, Actinin-associated LIM protein: identification of a domain interaction between PDZ and spectrin-like repeat motifs, J. Cell Biol. 139 (1997) 507–515.
[3] Q. Zhou, P. Ruiz-Lozano, M.E. Martone, J. Chen, Cypher, a striated muscle-restricted PDZ and LIM domain-containing protein, binds to alpha-actinin-2 and protein kinase C, J. Biol. Chem. 274 (1999) 19807–19813.
[4] M. Zheng, H. Cheng, I. Banerjee, J. Chen, ALP/Enigma PDZ–LIM domain proteins in the heart, J. Mol. Cell Biol. 2 (2010) 96–102.
[5] D. Frank, N. Frey, Cardiac Z-disc signaling network, J. Biol. Chem. 286 (2011) 9897–9904.
[6] S. Kuroda, C. Tokunaga, Y. Kiyohara, O. Higuchi, H. Konishi, K. Mizuno, G.N. Gill, U. Kikkawa, Protein-protein interaction of zinc finger LIM domains with protein kinase C, J. Biol. Chem. 271 (1996) 31029–31032.
[7] N. Nakagawa, M. Hoshijima, M. Oyasu, N. Saito, K. Tanizawa, S. Kuroda, ENH, containing PDZ and LIM domains, heart/skeletal muscle-specific protein, associates with cytoskeletal proteins through the PDZ domain, Biochem. Biophys. Res. Commun. 272 (2000) 505–512.
[8] A.D. Maturana, S. Walchli, M. Iwata, S. Ryser, J. Van Lint, M. Hoshijima, W. Schlegel, Y. Ikeda, K. Tanizawa, S. Kuroda, Enigma homolog 1 scaffolds protein kinase D1 to regulate the activity of the cardiac L-type voltage-gated calcium channel, Cardiovasc. Res. 78 (2008) 458–465.
[9] A. Lasorella, A. Iavarone, The protein ENH is a cytoplasmic sequestration factor for Id2 in normal and tumor cells from the nervous system, Proc. Natl. Acad. Sci. USA 103 (2006) 4976–4981.
[10] N. Niederlander, N.A. Fayein, C. Auffray, P. Pomies, Characterization of a new human isoform of the enigma homolog family specifically expressed in skeletal muscle, Biochem. Biophys. Res. Commun. 325 (2004) 1304–1311.
[11] J. Ito, T. Hashimoto, S. Nakamura, Y. Aita, T. Yamazaki, W. Schlegel, K. Takimoto, A.D. Maturana, Splicing transitions of the anchoring protein ENH during striated muscle development, Biochem. Biophys. Res. Commun. 421 (2012) 232–238.
[12] T. Yamazaki, S. Walchli, T. Fujita, S. Ryser, M. Hoshijima, W. Schlegel, S. Kuroda, A.D. Maturana, Splice variants of enigma homolog, differentially expressed during heart development, promote or prevent hypertrophy, Cardiovasc. Res. 86 (2010) 374–382.
[13] H. Cheng, K. Kimura, A.K. Peter, L. Cui, K. Ouyang, T. Shen, Y. Liu, Y. Gu, N.D. Dalton, S.M. Evans, K.U. Knowlton, K.L. Peterson, J. Chen, Loss of enigma homolog protein results in dilated cardiomyopathy, Circ. Res. 107 (2010) 348–356.
[14] T. Braun, M. Gautel, Transcriptional mechanisms regulating skeletal muscle differentiation, growth and homeostasis, Nat. Rev. Mol. Cell Biol. 12 (2011) 349–361.
[15] M.J. Thayer, S.J. Tapscott, R.L. Davis, W.E. Wright, A.B. Lassar, H. Weintraub, Positive autoregulation of the myogenic determination gene MyoD1, Cell 58 (1989) 241–248.