Biochemical and Biophysical Research Communications 444 (2014) 509–513

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The 31-kDa caspase-generated cleavage product of p130Cas antagonizes the action of MyoD during myogenesis

Da Eun Jeong [a,1], Eun Kyung Lee [b,1], Woo Keun Song [c,*], Wook Kim [a,*]

[a] Department of Molecular Science and Technology, Ajou University, Suwon 443-749, South Korea
[b] Department of Biochemistry, College of Medicine, The Catholic University of Korea, Seoul 137-701, South Korea
[c] Department of Life Science, Bio Imaging and Cell Dynamics Research Center, Gwangju Institute of Science and Technology, Gwangju 500-712, South Korea

ARTICLE INFO

Article history:
Received 13 January 2014
Available online 25 January 2014

Keywords:
p130Cas
31-kDa fragment
MyoD
bHLH
Myogenesis

ABSTRACT

Myogenesis is regulated by the basic helix-loop-helix (bHLH) myogenic regulatory factor MyoD, which induces muscle-specific gene expression by binding to the E-box sequence as a heterodimer with ubiquitous bHLH E2A (E12/E47) proteins. Here, we report that a 31-kDa caspase-generated cleavage product of Crk-associated substrate (p130Cas), herein called 31-kDa, is downregulated during muscle cell differentiation. 31-kDa contains a helix-loop-helix (HLH) domain that shows greater sequence homology with Id (inhibitor of DNA binding) proteins than with bHLH proteins. This HLH domain, lacking the basic region required for DNA binding, mediated the direct interaction of 31-kDa with MyoD. Overexpression of 31-kDa in C3H10T1/2 cells inhibited not only the transcriptional activation of $p21^{Waf1/Cip1}$ and E-box-dependent muscle-specific genes by MyoD and/or E2A but also MyoD-induced myosin heavy chain expression and myogenic conversion. In sum, our results suggest a role for 31-kDa as a negative regulator of MyoD in the muscle differentiation program.

1. Introduction

The 130-kDa adaptor molecule Crk-associated substrate (p130Cas) plays an important role in various cellular processes, including migration, proliferation, apoptosis, transformation, and invasion. It is composed of multiple protein–protein interaction domains, including a Src homology 3 domain, a proline-rich domain, a serine-rich domain, a Src-binding domain, and a large tyrosine kinase substrate (SD) domain. The SD domain contains 15 tyrosine-Xaa-Xaa-proline (YXXP) motifs that are the major sites of p130Cas tyrosine phosphorylation [1,2]. Tyrosine phosphorylation of p130Cas plays an essential role in various cellular responses, including receptor–receptor crosstalk [3,4]. We previously reported that the C-terminus of p130Cas contains a helix-loop-helix (HLH) domain that can mediate dimerization with another HLH protein [5]. During etoposide-induced apoptosis, caspase-mediated cleavage of p130Cas generates a C-terminal 31-kDa fragment (31-kDa) [5,6]. This cleavage is regulated by p130Cas tyrosine phosphorylation [7]. The phosphorylation of tyrosine and serine residues at the cleavage site confers resistance to caspase-3-mediated cleavage, and dephosphorylation of p130Cas precedes its cleavage during apoptosis [7].

31-kDa is highly conserved among proteins in the Cas family and promotes caspase-mediated cell death [5]. Sequence alignment of several HLH proteins and 31-kDa has revealed that 31-kDa contains a HLH domain lacking a basic region responsible for DNA binding. Further functional studies have shown that this domain mediates the formation of nonfunctional heterodimeric complexes with E12 and E47 [5], which are basic HLH (bHLH) transcription factors encoded by the *E2A* gene. These ubiquitously expressed E-proteins serve as partners within heterodimers with tissue-specific classes of bHLH proteins [8]. Once coupled to E12 and E47, 31-kDa is translocated into the nucleus, where it antagonizes their activity by sequestering them into an inactive state. This suggests that 31-kDa and bHLH transcription factors play opposing roles in the regulation of cell function [5]. 31-kDa has been shown to inhibit E2A-mediated transcription of $p21^{Waf1/Cip1}$, owing to the formation of the aforementioned inactive heterodimers. It has also been found that 31-kDa-mediated cell death is diminished by ectopic expression of $p21^{Waf1/Cip1}$, suggesting that 31-kDa serves as a dominant-negative inhibitor of bHLH transcription factors [5].

* Corresponding authors. Address: Department of Life Science, Bio Imaging and Cell Dynamics Research Center, Gwangju Institute of Science and Technology, 261 Cheomdan-gwagiro, Buk-gu, Gwangju 500-712, South Korea. Fax: +82 62 715 2484 (W.K. Song). Address: Department of Molecular Science and Technology, Ajou University, San 5, Woncheon-dong, Yeongtong-gu, Suwon 443-749, South Korea. Fax: +82 31 219 1610 (W. Kim).

E-mail addresses: wksong@gist.ac.kr (W.K. Song), wookkim21@ajou.ac.kr (W. Kim).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.01.085
0006-291X/

The bHLH family of transcription factors is involved in cell cycle regulation and cell fate specification, including skeletal muscle differentiation, and regulates the expression of tissue-specific target genes [9]. Differentiation of myogenic cells (myogenesis) is a well-characterized example, relying on tissue-specific bHLH transcription factors [10,11] and regulated by multiple factors acting either positively or negatively on the cells [12]. The most thoroughly characterized muscle-specific bHLH transcription factor is myoblast determination protein 1 (MyoD), also called myogenic factor 3 (Myf-3), which plays an important role in the commitment of a cell to the myogenic lineage and the initiation of myogenesis by coordinating muscle-specific gene expression [12,13]. MyoD-induced transcription of muscle-specific genes requires either homodimerization of MyoD, or heterodimerization with the gene products of the *E2A* gene, a process mediated by the HLH motif [8,14]. The homo- and heterodimers bind efficiently to the E-box, a consensus DNA sequence (CANNTG) that is a functionally important element in the upstream regulatory sequences of many muscle-specific genes [15]. The ability to bind the E-box as a homo- or heterodimer is regulated by the presence of negative regulators, including inhibitor of DNA binding (Id) proteins, a distinct subfamily of HLH proteins, while the ability of homo- or heterodimerization depends on the relative abundance of each transcription factor and/or the presence of other factors that inhibit dimerization [12,16]. The Id proteins can associate efficiently with MyoD and E2A to form nonfunctional heterodimers, impeding the ability of MyoD and E2A to bind the E-box, because Id proteins lack the basic region required for DNA binding. This suggests that Id proteins function as dominant-negative regulators of bHLH proteins, including MyoD and E2A [16].

As 31-kDa contains a HLH domain lacking a basic region and antagonizes E2A activity by forming a nonfunctional heterodimer [5], we speculated whether 31-kDa could also associate with MyoD and antagonize its function. In this study, we show that 31-kDa directly associates with MyoD and inhibits its function during myogenesis, similar to Id proteins.

2. Materials and methods

2.1. Cell culture, transfection, and luciferase assay

C2C12 and C3H10T1/2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Invitrogen) unless otherwise stated. E63 cells, myogenic clones of L8 rat skeletal myoblasts, were grown in DMEM supplemented with 10% horse serum (HS), 100 units/mL penicillin G, 100 μg/mL streptomycin sulfate, and 250 μg/mL amphotericin, as described previously [17]. To induce differentiation in E63 and C2C12 cells, the cells were transferred from growth medium to differentiation medium (DMEM, 2% HS).

Transient transfections were carried out using Lipofectamine Plus (Invitrogen) according to the manufacturers' instructions. C3H10T1/2 cells were transiently transfected with reporter and expression vectors encoding $p21^{Waf1/Cip1}$ promoter-luciferase (p21-Luc), troponin I promoter-luciferase (TnI-Luc), or muscle creatine kinase–luciferase (MCK–Luc). The amount of DNA in each transfection was equalized by the addition of empty parent vector. Following transfection, luciferase activity was determined using a luciferase assay system (Promega) and normalized to β-galactosidase activity.

2.2. Western blot analysis

Cells were lysed in modified RIPA buffer (50 mM Tris–HCl at pH 7.4, 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 10 mM NaF, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM sodium orthovanadate, 10 μM leupeptin, 1.5 μM pepstatin, and 10 μg/mL aprotinin). Proteins were separated by electrophoresis in 8–10% acrylamide gels and then analyzed by Western blot using anti-Cas antibody (BD Transduction Laboratories), anti-myosin heavy chain (MHC) antibody, or anti-tubulin monoclonal antibody (Sigma). Detection was performed using the ECL chemiluminescence kit (Amersham).

2.3. Immunoprecipitation

To detect interactions between 31-kDa and MyoD, C3H10T1/2 cells were transiently transfected with FLAG-tagged 31-kDa and MyoD. The cells were then lysed for 1 h at 4 °C in 1 mL of Triton X-100 buffer (10 mM Tris–HCl at pH 7.4, 5 mM EDTA, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 1 mM PMSF, 1 mM sodium orthovanadate, 10 μM leupeptin, 1.5 μM pepstatin, and 10 μg/mL aprotinin). After centrifugation, cell extracts were immunoprecipitated using anti-FLAG-M2 antibody (Sigma), and then analyzed by western blot using anti-MyoD antibody (Santa Cruz Biotechnology).

2.4. C3H10T1/2 cell myogenic conversion and immunofluorescence

To assay C3H10T1/2 cells for myogenic conversion, the cells were transiently transfected with expression vectors encoding the indicated proteins. Two days after transfection, cultures were transferred from growth to differentiation medium. Five days later, MHC expression was detected by immunofluorescence and Western blot analysis.

Immunostaining for MHC and MyoD was performed as described previously [4]. Cells were fixed, permeabilized, blocked, and incubated with anti-MHC antibody (Sigma) and anti-MyoD antibody (Santa Cruz Biotechnology), followed by staining with FITC- and TRITC-conjugated goat anti-mouse and anti-rabbit secondary antibodies (Invitrogen). Nuclei were visualized with Hoechst dye 33258 (Sigma). Images were obtained using a Leica fluorescence microscope (Leica Microsystems) and a CoolSNAPfx CCD camera equipped with MetaMorph imaging software (Molecular Devices).

2.5. Statistical analysis

Quantitative data are presented as the mean ± SEM. The differences between mean values were compared statistically by Student's *t*-test, using GraphPad Prism (GraphPad Software). A p value of <0.05 was considered statistically significant.

3. Results and discussion

3.1. 31-kDa is downregulated during myogenesis in culture

Speculating whether 31-kDa plays a role in myogenesis, we focused on MyoD as a possible target for inhibition by 31-kDa. To investigate the level of 31-kDa during differentiation of myogenic cells, we first examined the abundance of 31-kDa during differentiation of C2C12 cells, an immortalized mouse myoblast cell line derived from adult skeletal muscles [18]. The conversion of C2C12 myoblasts into post-mitotic multinucleated myotubes was induced by withdrawal of serum from the culture medium. The abundance of 31-kDa and MHC, a differentiation marker in C2C12 cells, was then analyzed by Western blot after 3, 5, and 7 days of serum withdrawal. Expression of MHC was upregulated under conditions in which the generation of 31-kDa was reduced, corresponding to the period of serum withdrawal (Fig. 1A). We further confirmed the reduction in the levels of 31-kDa using E63 cells

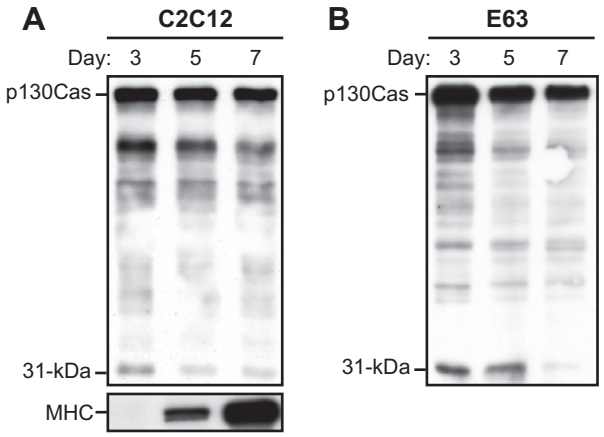

Fig. 1. The 31-kDa caspase-generated cleavage product (31-kDa) of Crk-associated substrate (p130Cas) is downregulated during skeletal muscle differentiation. Cell lysates were prepared from (A) C2C12 or (B) E6 cells after incubation in differentiation medium for 3, 5, and 7 days. The levels of p130Cas, 31-kDa, and a myogenesis marker (myosin heavy chain; MHC) were assessed by Western blot analysis.

(Fig. 1B). This expression pattern is similar to that of Id proteins during myogenesis [16,19]. Consistent with their negative regulatory role, the downregulation of Id proteins has been shown in myoblasts during myogenesis [16,19]. Conversely, the ectopic expression of Id proteins has been reported to inhibit the differentiation of muscle cells [19]. In addition, we have previously reported that caspase-mediated generation of 31-kDa is reduced by tyrosine phosphorylation of p130Cas at the cleavage site, and that conversely, dephosphorylation of p130Cas increases its cleavage [7]. Therefore, the reduction in 31-kDa is likely due to increased tyrosine phosphorylation of p130Cas during myogenesis [20]. Based on those findings, our results suggest that 31-kDa might not be required for the activation of muscle differentiation.

3.2. 31-kDa directly interacts with MyoD

To test whether 31-kDa associates with MyoD, C3H10T1/2 cells were transfected with expression vectors encoding FLAG-tagged 31-kDa and MyoD. Subsequently, cell lysates were subjected to co-immunoprecipitation using selected doses of anti-FLAG antibody, followed by Western blot analysis using anti-MyoD antibody. As expected, 31-kDa was found to associate with MyoD (Fig. 2A). To determine whether this association was direct and to identify which domain in 31-kDa mediated the association, we analyzed the ability of several 31-kDa deletion mutants to associate with MyoD, using in vitro glutathione S-transferase (GST) pull-down assays (Fig. 2B and C). Recombinant GST-fused 31-kDa mutant proteins were incubated with ^{35}S-labeled MyoD generated by in vitro transcription and translation. We found that the full-length 31-kDa, as well as its mutant proteins truncated at the C-terminus or N-terminus (GST-31ΔC and 31ΔN, respectively), were all able to pull down MyoD. However, MyoD did not bind to the GST protein (Fig. 2C), indicating a direct and specific association of 31-kDa with MyoD. Moreover, MyoD primarily bound to the HLH domain of 31-kDa truncated at the C- and N-termini (GST-31HLH) (Fig. 2C). This result demonstrates that the HLH domain of 31-kDa mediates the association with MyoD. Furthermore, as 31-kDa contains a HLH domain lacking the basic region required for DNA-binding, the data strongly suggest that 31-kDa acts as a dominant-negative regulator of MyoD by forming inactive heterodimers.

3.3. 31-kDa inhibits MyoD-dependent gene activation

In light of the inhibitory activity of 31-kDa, we next examined whether it might function as a transcriptional repressor of MyoD. Cell cycle exit, characterized by the activation of the cell cycle inhibitor $p21^{Waf1/Cip1}$, is required for differentiation of muscle cells and is triggered by MyoD-induced expression of $p21^{Waf1/Cip1}$ [21]. Hence, we performed a reporter gene assay, using a luciferase construct, to test the effect of 31-kDa on MyoD-induced transcription of *$p21^{Waf1/Cip1}$*. The p21-Luc construct contains eight putative E-boxes and is transactivated by MyoD and E2A [5,21,22]. In agreement with previous reports [5,21], we found that p21-Luc was activated to high expression levels by either MyoD or E47, as well as by the co-expression of MyoD and E47 (Fig. 3A). In contrast, 31-kDa showed no transcriptional activity on its own, and similar to Id2 proteins, interfered with the ability of MyoD and E47 to activate transcription (Fig. 3A).

We next examined the inhibitory effects of 31-kDa on the MyoD-activated, myogenic-specific transcription of the troponin I and muscle creatine kinase promoters. As expected, both MyoD and E47 produced increased levels of the TnI-Luc and MCK-Luc constructs, and their activity levels were further increased by the co-expression of MyoD and E47 (Fig. 3B and C). Similar to Id2 proteins, 31-kDa significantly repressed MyoD- or E47-induced transcriptional activation of TnI-Luc and MCK-Luc (Fig. 3B and C). Taken together, our results suggest that 31-kDa acts as a powerful transcriptional repressor of MyoD.

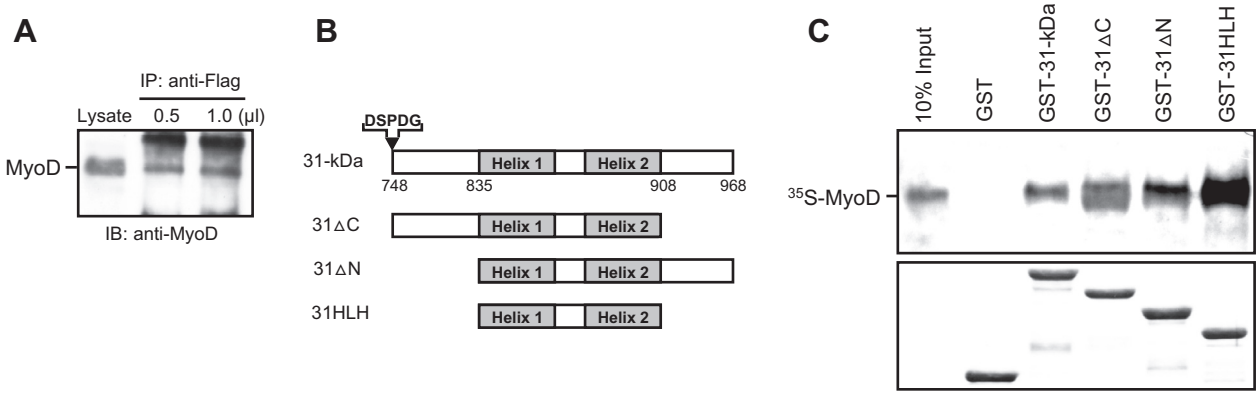

Fig. 2. Association of the 31-kDa with MyoD. (A) C2C12 cells were transfected with expression plasmids containing FLAG-tagged 31-kDa. Cell lysates were then immunoprecipitated with anti-FLAG antibody and analyzed by Western blot using anti-MyoD antibody. (B) Schematic representation of various 31-kDa mutants. (C) Interaction of ^{35}S-labeled, in vitro translated MyoD with glutathione S-transferase (GST)-fusion mutant proteins of 31-kDa. The Coomassie Blue-stained gel in the lower panel confirms the integrity and equal loading of the GST-fusion proteins.

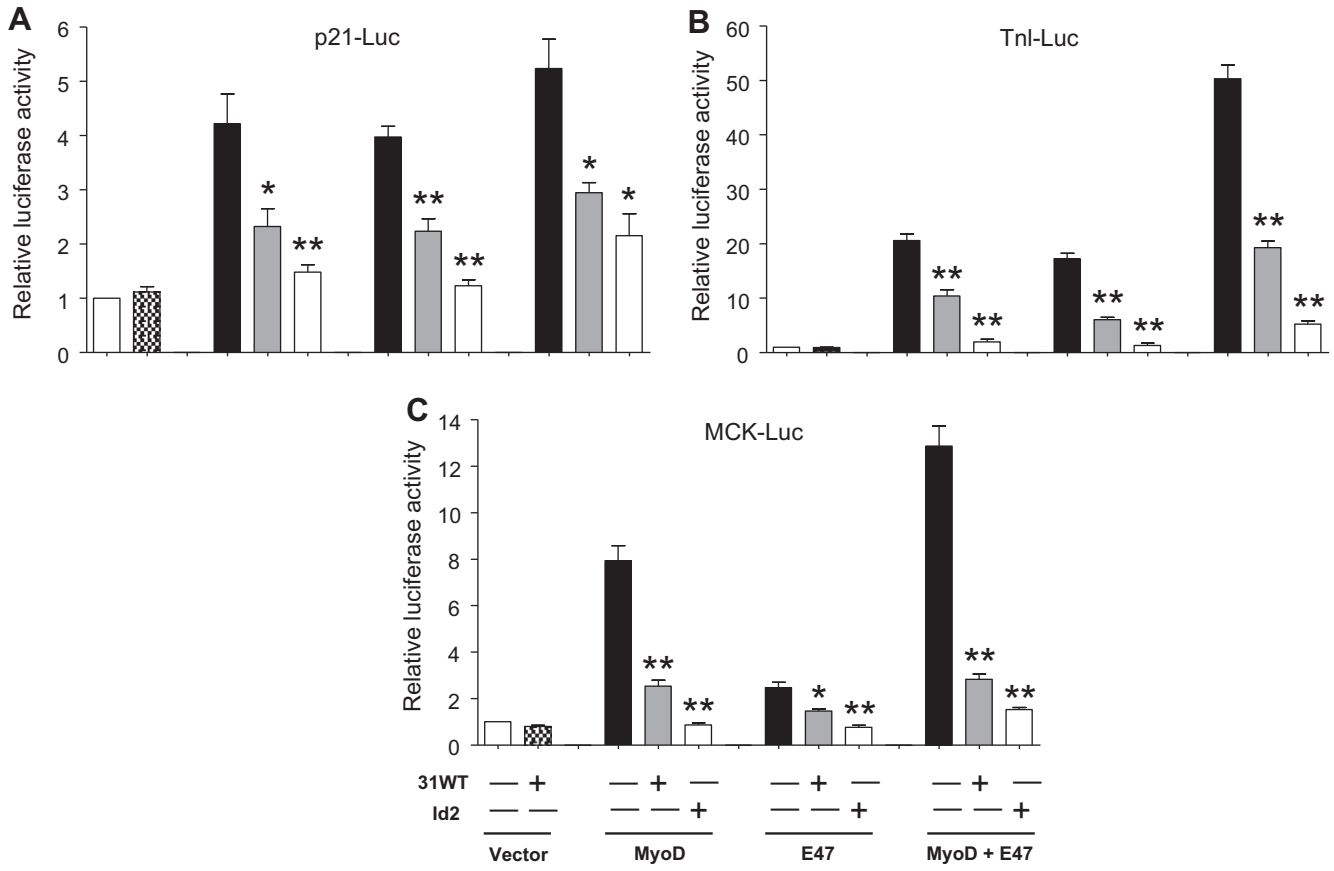

Fig. 3. The 31-kDa represses MyoD-mediated transcriptional activation of muscle-specific gene expression. C3H10T1/2 cells were transiently transfected with reporter and expression vectors encoding (A) $p21^{Waf1/Cip1}$ promoter-luciferase ($p21^{Waf1/Cip1}$-Luc), (B) troponin I promoter-luciferase (Tnl-Luc), or (C) muscle creatine kinase–luciferase (MCK–Luc). The amount of DNA in each transfection was kept constant by the addition of empty vector. Luciferase activity was measured 36 h after transfection and normalized to the activity of β-galactosidase. Data are shown as the mean ± SEM from three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$.

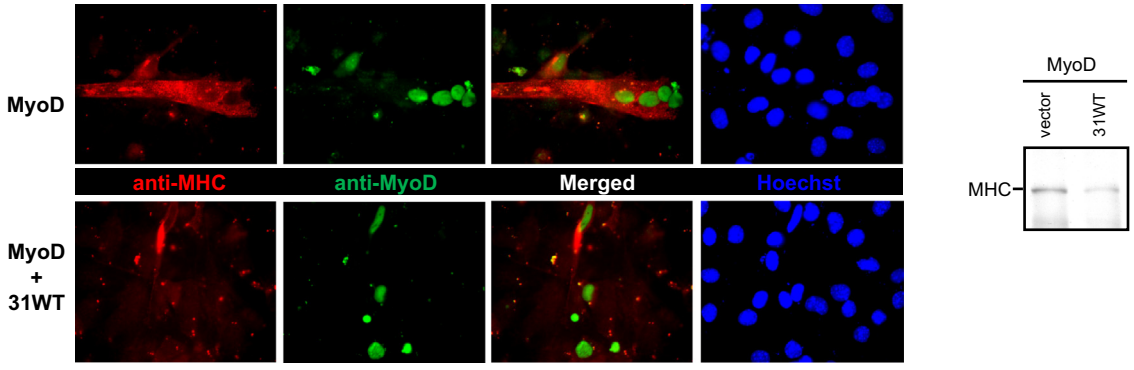

Fig. 4. The 31-kDa represses MyoD-induced expression of MHC and myotube formation. C3H10T1/2 cells transfected with expression vectors encoding MyoD and/or 31-kDa were incubated for 5 days in differentiation medium. The amount of DNA used for transfection was adjusted with empty vector. MHC and MyoD expression was analyzed by immunofluorescence staining and Western blot analysis.

Using an electrophoretic mobility shift assay in an earlier study, we found that 31-kDa represses the transcriptional activity of E2A by forming inactive heterodimers incapable of binding to the E-box [5]. Thus, the most likely explanation for our new results is that 31-kDa prevents MyoD from forming functionally active homodimers, or heterodimers with E2A. When functionally active, such dimers bind with high affinity to the $p21^{Waf1/Cip1}$ promoter and the muscle-specific enhancers, and positively regulate their expression, leading to cell cycle arrest and commitment to muscle differentiation.

3.4. 31-kDa blocks the ability of MyoD to activate myogenesis

We finally tested whether 31-kDa inhibits MyoD-induced MHC expression and myotube formation in C3H10T1/2 cells. C3H10T1/2 cells transiently transfected with expression vectors encoding MyoD with or without 31-kDa were transferred from growth to differentiation medium, and MHC expression was detected by immunofluorescence and Western blot analysis. As shown in Fig. 4, MyoD induced MHC expression and myotube formation in C3H10T1/2 cells. When 31-kDa was expressed together with

MyoD, however, the ability of MyoD to induce MHC expression and myotube formation was inhibited, suggesting that 31-kDa acts as a negative regulator of MyoD-induced myogenesis.

The finding that 31-kDa is an inhibitor of MyoD-induced differentiation of muscle cells and is downregulated in the early steps of the myogenic pathway is similar to the finding that Id proteins act as negative regulators of MyoD and E2A to block the activation of muscle-specific genes and muscle cell differentiation [16,19]. Therefore, our results demonstrate another mechanism by which 31-kDa inhibits muscle cell differentiation by forming inactive heterodimers with MyoD or E2A. Providing the cells with an additional level of control in the network of bHLH protein regulators of myogenesis, this regulatory mechanism may operate to control cell cycle exit, commitment to cell lineages, and the timing of differentiation.

Acknowledgments

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Korean Ministry of Education (No. 2009-0093826). W.K.S. was supported by the Bio Imaging and Cell Dynamics Research Center, funded by the Korean Ministry of Science, ICT and Future Planning (MSIP) (2007-0056157). E.K.L. was supported by the National Research Foundation of Korea (NRF), funded by the Korean MSIP (2012M3A9D1054476).

References

[1] R. Sakai, A. Iwamatsu, N. Hirano, S. Ogawa, T. Tanaka, H. Mano, Y. Yazaki, H. Hirai, A novel signaling molecule, p130, forms stable complexes in vivo with v-Crk and v-Src in a tyrosine phosphorylation-dependent manner, EMBO J. 13 (1994) 3748–3756.

[2] D. Chodniewicz, R.L. Klemke, Regulation of integrin-mediated cellular responses through assembly of a CAS/Crk scaffold, Biochim. Biophys. Acta 1692 (2004) 63–76.

[3] W. Kim, Y.S. Kang, J.S. Kim, N.Y. Shin, S.K. Hanks, W.K. Song, The integrin-coupled signaling adaptor p130Cas suppresses Smad3 function in transforming growth factor-beta signaling, Mol. Biol. Cell 19 (2008) 2135–2146.

[4] Y.S. Kang, D.E. Jeong, E.K. Lee, W.K. Song, W. Kim, P130Cas controls the susceptibility of cancer cells to TGF-β-induced growth inhibition, Biochem. Biophys. Res. Commun. 438 (2013) 116–121.

[5] W. Kim, S. Kook, D.J. Kim, C. Teodorof, W.K. Song, The 31-kDa caspase-generated cleavage product of p130cas functions as a transcriptional repressor of E2A in apoptotic cells, J. Biol. Chem. 279 (2004) 8333–8342.

[6] S. Kook, S.R. Shim, S.J. Choi, J. Ahnn, J.I. Kim, S.H. Eom, Y.K. Jung, S.G. Paik, W.K. Song, Caspase-mediated cleavage of p130cas in etoposide-induced apoptotic Rat-1 cells, Mol. Biol. Cell 11 (2000) 929–939.

[7] D.H. Kim, S.J. Choi, S. Kook, W. Kim, W.K. Song, Phosphorylation-dependent cleavage of p130cas in apoptotic rat-1 cells, Biochem. Biophys. Res. Commun. 300 (2003) 141–148.

[8] A.B. Lassar, R.L. Davis, W.E. Wright, T. Kadesch, C. Murre, A. Voronova, D. Baltimore, H. Weintraub, Functional activity of myogenic HLH proteins requires hetero-oligomerization with E12/E47-like proteins in vivo, Cell 66 (1991) 305–315.

[9] B.A. Westerman, C. Murre, C.B. Oudejans, The cellular Pax-Hox-helix connection, Biochim. Biophys. Acta 1629 (2003) 1–7.

[10] K. Yun, B. Wold, Skeletal muscle determination and differentiation: story of a core regulatory network and its context, Curr. Opin. Cell Biol. 8 (1996) 877–889.

[11] J.D. Molkentin, E.N. Olson, Defining the regulatory networks for muscle development, Curr. Opin. Genet. Dev. 6 (1996) 445–453.

[12] C.A. Berkes, S.J. Tapscott, MyoD and the transcriptional control of myogenesis, Semin. Cell Dev. Biol. 16 (2005) 585–595.

[13] L. Kassar-Duchossoy, B. Gayraud-Morel, D. Gomès, D. Rocancourt, M. Buckingham, V. Shinin, S. Tajbakhsh, Mrf4 determines skeletal muscle identity in Myf5:Myod double-mutant mice, Nature 431 (2004) 466–471.

[14] J.S. Hu, E.N. Olson, R.E. Kingston, HEB, a helix-loop-helix protein related to E2A and ITF2 that can modulate the DNA-binding ability of myogenic regulatory factors, Mol. Cell. Biol. 12 (1992) 1031–1042.

[15] C. Murre, P.S. McCaw, H. Vaessin, M. Caudy, L.Y. Jan, Y.N. Jan, C.V. Cabrera, J.N. Buskin, S.D. Hauschka, A.B. Lassa, H. Weintraub, D. Baltimore, Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence, Cell 58 (1989) 537–544.

[16] R. Benezra, R.L. Davis, D. Lockshon, D.L. Turner, H. Weintraub, The protein Id: a negative regulator of helix-loop-helix DNA binding proteins, Cell 61 (1990) 49–59.

[17] S.J. Kaufman, C.M. Parks, Loss of growth control and differentiation in the fu-1 variant of the L8 line of rat myoblasts, Proc. Natl. Acad. Sci. USA 74 (1977) 3888–3892.

[18] D. Yaffe, O. Saxel, Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle, Nature 270 (1977) 725–727.

[19] Y. Jen, H. Weintraub, R. Benezra, Overexpression of Id protein inhibits the muscle differentiation program: in vivo association of Id with E2A proteins, Genes Dev. 6 (1992) 1466–1479.

[20] K. Kawauchi, W.W. Tan, K. Araki, F.B. Abu Bakar, M. Kim, H. Fujita, H. Hirata, Y. Sawada, p130Cas-dependent actin remodelling regulates myogenic differentiation, Biochem. J. 445 (2012) 323–332.

[21] O. Halevy, B.G. Novitch, D.B. Spicer, S.X. Skapek, J. Rhee, G.J. Hannon, D. Beach, A.B. Lassar, Correlation of terminal cell cycle arrest of skeletal muscle with induction of p21 by MyoD, Science 267 (1995) 1018–1021.

[22] S. Prabhu, A. Ignatova, S.T. Park, X.H. Sun, Regulation of the expression of cyclin-dependent kinase inhibitor p21 by E2A and Id proteins, Mol. Cell. Biol. 17 (1997) 5888–5896.