Pctaire1/Cdk16 promotes skeletal myogenesis by inducing myoblast migration and fusion

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Abstract
The Cdk-related protein kinase Pctaire1/Cdk16 is abundantly expressed in brain, testis and skeletal muscle. Functional roles of Pctaire1 such as regulation of neuron migration and neurite outgrowth thus far have been mainly elucidated in the field of nervous system development. Although these regulations based on cytoskeletal rearrangements evoke a possible role of Pctaire1 in the development of skeletal muscle, little is known in this regard. In this study, we demonstrated that myogenic differentiation and subsequent fusion is promoted in Pctaire1 overexpressing cells, and conversely, is inhibited in the knockdown cells. Furthermore, our findings suggest that Pctaire1 exerts promyogenic effects by regulating myoblast migration and process formation during skeletal myogenesis.

1. Introduction
Myogenic differentiation is an essential process for muscle growth and homeostasis during embryonic development and in postnatal life. Myogenesis is accomplished by undergoing multistep process accompanied by a complex series of molecular and morphological changes. After proliferation, followed by cell cycle withdrawal, mononucleated myoblasts differentiate into elongated myocytes, then migrate, adhere and fuse to one another to form multinucleated myotubes, and mature muscle fiber in the end [1–3]. The myogenic program is orchestrated by sequential activation of myogenic regulatory factors (MRFs), the basic helix–loop–helix (bHLH) family of transcription factors (Myf5, MyoD, myogenin and MRF4), which can induce expression of muscle-specific genes in concert with transcriptional cofactors such as Mef2 and E proteins [4–9].

Accumulating evidence suggests that protein kinases functioning as substrates for caspase-3 play a key role in regulating differentiation [10–13]. As previously reported, we have identified 30 protein kinases as novel substrates for caspase-3 [14], and investigated their function in skeletal muscle differentiation [13]. In consequence, we could pick out Pctaire1 kinase as one of the positive regulators of myogenic differentiation as in the case of previous report regarding Nek5 [13].

A serine/threonine kinase Pctaire1 (now known as Cdk16) is one of the atypical members in cyclin-dependent kinase (Cdk) family, and has both N- and C-terminal extensions that are divergent from or absent in other Cdks. These additional domains play important roles in mediating protein–protein interactions and were shown to regulate kinase activity [15–17]. Activity of Cdks is generally regulated by binding to an activating subunit, cyclin. Unlike conventional cyclin-Cdk interactions, however, not only the catalytic domain but also domains within the N-terminal extension of Pctaire1 are required for binding to its activator cyclin Y [18]. Although Pctaire1 activity has been shown as cell cycle regulated, it remains uncertain whether Pctaire1 itself is involved in cell cycle progression in the same way as some other Cdks [17,19]. Also, an alternative regulatory mechanism has been proposed to activate and inhibit the kinase activity of Pctaire1 through phosphorylation by Cdk5/p35 and protein kinase A (PKA), respectively [16,20].

Pctaire1 is ubiquitously expressed in mammalian tissues including skeletal muscle and is particularly abundant in terminally differentiated cells in brain and testis, and in transformed cell...
lines [16,20–22]. During development of mouse brain, Pctaire1 cooperates with Cdk5 to initiate a kinase cascade that governs cytoskeletal rearrangements essential for neuron migration and neurite outgrowth by the myocardin-related transcription factors (MRTFs)/serum response factor (Srf) pathway [23]. In human cell lines, Pctaire1 is phosphorylated at several residues, including Ser119 and Ser153, which have been shown to be substrates for PKA in vitro [16,17]. Phosphorylation of Ser119 and Ser153 creates 14-3-3 consensus binding motifs, and alanine mutation of both residues abolishes the binding of Pctaire1 to 14-3-3 in the presence of active PKA, which also results in the inhibition of neurite outgrowth in Neuro-2A cells [16]. However, the precise role of this modification and 14-3-3 binding remains to be elucidated. In addition to the function in the nervous system, by generating a conditional Pctaire1-knockout mouse, Mikolcevic et al. found that Pctaire1 is essential for the completion of spermatogenesis [17]. Moreover, Pctaire1 has also been reported to be involved in the formation of myoblasts. Pctaire1 is essential for the completion of spermatogenesis [17]. Moreover, Pctaire1 has also been reported to be involved in the regulation of intracellular vesicles and secretion [24,25].

In this study, we focus on the process of myogenesis in which the role of Pctaire1 remains to be defined though its functions involved with cytoskeletal rearrangements and exocytosis in nervous system are linked to the process of muscle cell fusion [26]. We report here, Pctaire1 is involved in the promotion of myogenic differentiation, and fusion by inducing migration and process formation of myoblasts.

2. Materials and methods

2.1. Antibodies

Antibodies against the following antigens were used: myosin heavy chain (MHC) (clone MF20; anti-all isoforms, Developmental Studies Hybridoma Bank, Iowa City, IA), troponin C (sc-20642, Santa Cruz Biotechnology, Santa Cruz, CA), Pctaire1 (#4852, Cell Signaling Technology, Beverly, MA), V5 epitope (Invitrogen, Carlsbad, CA), cleaved caspase-3 (#9664, clone 5A1E, Cell Signaling Technology), Pctaire1 (#4852, Cell Signaling Technology) and α-tubulin (clone DM1A, Sigma, St. Louis, MO).

2.2. Plasmid constructs

To construct expression plasmids for mammalian cells, cDNAs encoding human MyoD1 (RefSeq: NM_002478), human PCTAIRE1 (RefSeq: NM_006201) and mouse Pctaire1 (RefSeq: NM_011049) were subcloned into vectors (pcDNA3.1/nV5-DEST, pcDNA3.2/V5-DEST, and pcDNA6.2/N-EmGFP-DEST) (Invitrogen). Mutants of human PCTAIRE1 were generated by site-directed mutagenesis using a PrimeSTAR mutagenesis basal kit (Takara Bio Inc., Otsu, Japan).

2.3. Cell culture and transfection

C2/4 [27] is a subclone of C2C12 mouse myoblast cell line, and is referred to simply as C2C12 herein. C2C12 cells were maintained at 37°C in a 5% CO2 incubator in a growth medium (GM) of Dulbecco’s modified eagle medium (DMEM) (Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO), 2 mM l-glutamine (GIBCO, Grand Island, NY), and antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin) (GIBCO), as previously reported [13]. Myogenic differentiation of C2C12 myoblasts was induced by replacing the GM with a differentiation medium (DM) that is DMEM with high glucose (Wako, Osaka, Japan) supplemented with 2% horse serum and penicillin-streptomycin. Unless otherwise noted, cells were cultured in GM, and all transfections in this study were performed in a semi-confluent condition using TransIT-LT1 transfection reagent (Mirus, Madison, WI) according to the manufacturer’s instruction.

2.4. Western blot analysis

All Western blot analyses were performed as described previously [28]. Briefly, proteins in whole-cell lysates were separated by SDS–PAGE and transferred onto a PVDF membrane by electroblotting. After blocking with 5% milk/TBST, the membrane was probed with a given primary antibody and then incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody. The immunoreactive proteins were visualized using Immobilon Western HRP substrate Luminol Reagent (Millipore, Bedford, MA) and LAS-4000 mini biomolecular imager (GE Healthcare, Piscataway, NJ).

2.5. Immunostaining analysis

Cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 15 min at room temperature, and then permeabilized with 0.5% Triton X-100 in PBS for 5 min. After blocking with 5% calf serum in TBST for 1 h, the cells were incubated with anti-troponin C antibody, anti-MHC antibody or anti-V5 antibody for 1 h at 37°C. The cells were then incubated with Alexa Fluor 488- and/or 568-conjugated appropriate secondary antibodies (Molecular Probes, Eugene, OR) for 1 h at room temperature. DAPI staining was carried out concomitantly with the above procedure. Fluorescence images were acquired with the Carl Zeiss LSM710 confocal laser scanning microscope (Carl Zeiss, Jena, Germany).

2.6. Real-time quantitative RT-PCR analysis

Total cellular RNA was extracted from C2C12 cells using a QIA-easy RNA mini kit (Qiagen, Valencia, CA). Extracted total RNA was subjected to reverse transcription with a Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel, Schweiz). Real-time qRT-PCR analysis was performed using a LightCycler 480 Real-time PCR System (Roche). The following primer sets were designed by the software of Assay Design Center (Roche): 5′-GGCAATCTGG ATCTCTGCT-3′ (forward) and 5′-CCCTCCTATGCAGCAACAAATGGAAA-3′ (reverse) for Pctaire1; 5′-TGAAGAATGATGGATTTTATTTTCTTAAGC-3′ (forward) and 5′-GACGACACATGGAAGTTCTGAGG-3′ (reverse) for Me2a; and 5′-CTGCTCCCTAGCAGTGTTGC-3′ (forward) and 5′-GGTTGTTGGTGTAATCAGT-3′ (reverse) for Me2c. The values were normalized to GAPDH (primer set was purchased from Roche).

2.7. Lentivirus-mediated gene transfer and generation of stable cell lines

Lentivirus-mediated RNA interference (RNAi) was utilized for the generation of stable cell lines in which the expression of Pctaire1 is suppressed, and followed by knockdown analysis. For this purpose, an HIV-based self-inactivating lentiviral expression vector (RIKEN BioResource Center, Japan [29]) was slightly modified by adding a transgene cassette to drive the expression of a short hairpin RNA (shRNA), and the resulting expression vector was termed as CSII-CMV-GFP-IRES-Bsd-H1 [30]. In this study, the short hairpin RNA (shRNA), and the resulting expression vector was termed as CSII-CMV-GFP-IRES-Bsd-H1 [30]. In this study, the short hairpin RNA (shRNA), and the resulting expression vector was termed as CSII-CMV-GFP-IRES-Bsd-H1 [30]. In this study, the short hairpin RNA (shRNA), and the resulting expression vector was termed as CSII-CMV-GFP-IRES-Bsd-H1 [30]. In this study, the short hairpin RNA (shRNA), and the resulting expression vector was termed as CSII-CMV-GFP-IRES-Bsd-H1 [30]. In this study, the short hairpin RNA (shRNA), and the resulting expression vector was termed as CSII-CMV-GFP-IRES-Bsd-H1 [30]. In this study, the short hairpin RNA (shRNA), and the resulting expression vector was termed as CSII-CMV-GFP-IRES-Bsd-H1 [30]. In this study, the short hairpin RNA (shRNA), and the resulting expression vector was termed as CSII-CMV-GFP-IRES-Bsd-H1 [30]. In this study, the short hairpin RNA (shRNA), and the resulting expression vector was termed as CSII-CMV-GFP-IRES-Bsd-H1 [30]. In this study, the short hairpin RNA (shRNA), and the resulting expression vector was termed as CSII-CMV-GFP-IRES-Bsd-H1 [30]. In this study, the short hairpin RNA (shRNA), and the resulting expression vector was termed as CSII-CMV-GFP-IRES-Bsd-H1 [30]. In this study, the short hairpin RNA (shRNA), and the resulting expression vector was termed as CSII-CMV-GFP-IRES-Bsd-H1 [30]. In this study, the short hairpin RNA (shRNA), and the resulting expression vector was termed as CSII-CMV-GFP-IRES-Bsd-H1 [30].
transmission of the transgene, a pool of the C2C12 cells resistant to blasticidin S (10 μg/ml, Invitrogen) was generated and used in subsequent analysis.

2.8. Wound-healing migration assay

Confluent cultures of C2C12 cells were scraped off with a pipette tip to obtain an acellular area, and GM was then replaced to remove floating cells. Phase contrast images of scraped area were acquired with a CKX41 microscope (Olympus, Japan), and the number of cells that migrated into the area was counted after 6 h.

2.9. Measurement of cell length

Twenty-four hours after transfection with GFP-fusion protein expression vectors, semi-confluent C2C12 cells were fixed, and then stained with DAPI. Three fields were randomly chosen for acquisition of fluorescent and phase contrast images. Cell length of GFP-positive cells was then measured on these images using ZEN imaging software (Carl Zeiss, Jena, Germany). The cell length in this analysis was defined as a sum of the longest distance from the center of nucleus to each of two points.

2.10. Statistical analysis

Bar graph data are mean ± S.D. Student’s t-test was used to determine the significance of differences, unless otherwise noted. Dunnett’s multiple comparison test was used in Fig. 5A. P values of less than 0.05 were considered statistically significant.

2.11. ChIP-seq analysis

ChIP-seq data (GEO accession number GSE44824) for MyoD and myogenin were used in this study. To find the binding peaks, the data sets (RedGraph format) were visualized by Integrated Genome Browser (IGB).

3. Results and discussion

3.1. Pctaire1 promotes myogenic differentiation of C2C12 myoblasts

We recently identified 30 protein kinases including Pctaire1 as novel substrates for caspase-3 [14], and have investigated the role of them in skeletal muscle differentiation as in the case of previous report regarding Nek5 [13]. In consequence of the overexpression analysis, the promyogenic effect of human Pctaire1 (indicated by a capital letter in Figure) was found in C2C12 cells as increase in the expression levels of two myogenic markers: myosin heavy chain (MHC) and troponin C (Fig. 1A). The equal promyogenic effect of mouse Pctaire1 that shares 97.6% homology (including key residues: Ser119, Ser153 and Lys194) with human was also confirmed by comparison with EGFP-V5 (transfection control) and MyoD1, a muscle-specific regulator, which is known to activate the myogenic differentiation program. Furthermore, immunostaining showed that most cells expressing exogenous Pctaire1 were differentiated cells that were positive for troponin C expression, though the size of myotubes is inferior to that of MyoD1 (Fig. 1B). In a previous report on another caspase-3 substrate from the same screening, we demonstrated that cleavage of Nek5 occurs when caspase-3 activity reaches the peak level after induction of myogenic differentiation in C2C12 cells, and Nek5 or caspase-3-cleaved Nek5 exerts a promyogenic effect through up-regulation of caspase-3 activity [13]. Thus, we next investigated the connection with caspase-3 to gain insight into Pctaire1 function as a caspase-3 substrate. Although in vitro experiments revealed that Pctaire1 is cleaved by caspase-3 (Supplementary Fig. S1) [14], the cleavage of Pctaire1 in C2C12 cells was not observed during myogenic differentiation under the same condition in which Nek5 cleavage occurs (Fig. 1C). Moreover, no significant difference in the caspase-3 activity was found by overexpression between EGFP-V5 and Pctaire1 (Fig. 1C). Together, these findings indicate that Pctaire1 is able to promote myogenic differentiation and the function in myogenesis is independent of caspase-3.

3.2. Up-regulation of Pctaire1 expression occurs during skeletal muscle differentiation

Pctaire1 is known to be prominently expressed in post-mitotic cells such as in brain and testis [21,22], and here, we monitored the expression levels of Pctaire1 to investigate its regulation during skeletal muscle differentiation. Pctaire1 transcript levels were increased under conditions of both growth (GM) and differentiation (DM) (Fig. 2A). In GM, differentiation of C2C12 myoblasts seems to be induced by high cell density-induced cell-cycle withdrawal. In fact, Mef2a and Mef2c, major cofactors of MRFs, were induced in GM as well as DM (Supplementary Fig. S2). Additionally, time-dependent accumulation of Pctaire1 protein is observed prior to dramatic increase in the levels of MHC protein (Fig. 2B). These results indicate that skeletal muscle differentiation is accompanied by up-regulation of Pctaire1. In support of this notion, publicly available ChIP-seq data (GEO accession number GSE44824) from C2C12 cells that were differentiated in similar DM for 60 h revealed binding of MyoD and myogenin in close proximity to the transcription start site of Pctaire1 (Supplementary Fig. S3), suggesting the possibility that the myogenic transcription factors directly regulate Pctaire1 expression during differentiation of C2C12 cells.

3.3. Suppression of Pctaire1 expression blocks the process of skeletal myogenesis

Next, to assess whether up-regulation of Pctaire1 is indeed required for the progression of myogenic differentiation, we established C2C12 cells stably expressing short hairpin RNA (shRNA) targeting mouse Pctaire1 (shPctaire1-#1 and shPctaire1-#2), in which the levels of Pctaire1 mRNA and protein were significantly decreased compared with cells expressing non-target shRNA (shNC) or shLacZ (Fig. 3A and B). We then investigated the effect of Pctaire1 knockdown on C2C12 differentiation and found that expression levels of muscle specific genes including MHC and troponin C were remarkably reduced in Pctaire1 knockdown cells (Fig. 3B). Furthermore, MHC immunostaining revealed that Pctaire1-deficient cells display obvious defects of myogenic differentiation (Fig. 3C, left panel). To confirm this observation, we quantified the degree of myotube formation by counting the number of nuclei in each knockdown cell. As expected, a large number of mononucleated cells were noted in Pctaire1 knockdown cells (Fig. 3C, right graph). Together, these results strongly suggest that Pctaire1 expression during skeletal muscle differentiation contributes to the progression of myogenesis.

3.4. Suppression of Pctaire1 expression inhibits myoblast migration

It was recently reported that Pctaire1 plays an important role in the control of cytoskeletal rearrangements that are essential for neuronal migration and neurite outgrowth during brain development [23,31]. Although these functional roles of Pctaire1 thus far have been characterized mainly in nervous system, which seems to be coincidentally associated with the myogenesis because cell migration and formation of a large F-actin-based protrusion is fundamental aspects of the process of myoblast fusion [2].
Thus, to define the possible role of Pctaire1 in skeletal myogenesis, we primarily examined its effect on myoblast migration using in vitro wound-healing assay. Myoblast migration into scraped area was observed and quantified by calculating the percentage of the number of cells that migrated relative to that of cells stably expressing negative control shRNA (Fig. 4A and B). The result
Fig. 3. Knockdown of Pctaire1 blocks the process of skeletal myogenesis. C2C12 cells stably expressing indicated shRNAs were used in the following experiments. (A) C2C12 cells were cultured in GM or DM for 3 days after confluence, and then collected. The relative expression levels of Pctaire1 mRNA were determined by real-time quantitative RT-PCR. Error bars indicate S.D. (N = 4). (B) C2C12 cells were cultured in DM for 3 days after confluence. The cell lysates were subjected to Western blot analysis. (C) Differentiated cells were immunostained with an anti-MHC antibody (red) after 4 days of culture in DM. The number of nuclei in the indicated shRNA-expressing cells (EGFP positive) was counted by DAPI staining (blue) and grouped into three categories (1, 2–9, and 10<), and then the percentage of that was quantified (right graph). Error bars indicate S.D. (N = 3, each N consists of 100 < cells). Statistical analysis: ***P < 0.001 vs control (shNC), §§§ P < 0.001 vs control (shLacZ), and §§ P < 0.005 vs control (shLacZ), at a group of mononucleated cells.

Fig. 4. Knockdown of Pctaire1 inhibits myoblast migration. Wound-healing migration assay was performed using C2C12 cells stably expressing indicated shRNAs. (A) Scraped area (between lines on phase contrast images) was observed at different time. (B) The number of cells that migrated into the scraped area was counted after 6 h. Results were expressed as a percentage of control cells (shNC). Error bars indicate S.D. (N = 4). Statistical analysis: **P < 0.005 vs control (shNC), §§P < 0.001 vs control (shLacZ), and §P < 0.01 vs control (shLacZ).
showed that suppression of Pctaire1 expression inhibits the migration of C2C12 cells, suggesting that the involvement of Pctaire1 in migration is required for the fusion process.

3.5. Pctaire1-induced process formation promotes fusion of skeletal muscle cell

To gain further insights into how Pctaire1 is involved in promoting myogenesis, we next asked whether Pctaire1 promotes process formation like neurite outgrowth in C2C12 cells. For assessment of morphological change, cells cultured in GM for 1 day after transfection were observed in semi-confluent condition. The process formation as observed in neuronal cell was induced in C2C12 cells overexpressing wild-type Pctaire1 (WT) compared to EGFP-V5 as a control (Fig. 5A). Additionally, their cell length was measured and quantified. The same phenotype as WT was also observed in cells overexpressing kinase-dead mutant (K194A), suggesting that it is independent of kinase activity of Pctaire1. As previously reported, two alanine mutants of serine residue, S119A and S119A/S153A, displayed resistance to the process formation compared with WT. We then assessed the efficiency of myotube formation of cells expressing WT-Pctaire1 and its mutants to investigate whether differences of cell length as shown in Fig. 5A affect the fusion. Interestingly, the result showed that the cell length correlated with the percentage of multinucleated cells (Fig. 5B). Most importantly, the result suggests that cells with an elongated shape are more able to proceed with the fusion efficiently. This may result from increased opportunity of elongated cells for contact with cells that are competent to fuse. In addition, the factor of cell length seems to contribute to the fusion process rather than differentiation process, because the myogenic differentiation in itself was certainly promoted by S119A and S119A/S153A mutants compared with those effects on the fusion (Supplementary Fig. S4).

In human cell lines, Pctaire1 is phosphorylated at several residues, including Ser119 and Ser153 [16]. Phosphorylation of S119 and S153 is considered to be a key event for binding to 14-3-3 proteins including γ and ζ isoform, since alanine mutation of either residue is sufficient to abolish its ability to bind [16]. It has been reported that 14-3-3 isoforms (γ, ζ) interact with phosphorylated slingshot phosphatases (SSH1, 2 and 3) [32–34], which is considered to lead to inhibition of SSH activity [33]. Moreover, in cells stimulated with neuregulin, expression of 14-3-3γ prevents translocation of both SSH1 and cofilin to lamellipodia and inhibits cofilin dephosphorylation [33,35]. Cofilin, one of F-actin-severing proteins, plays a crucial role in the regulation of actin polymerization accompanied by dynamic changes in the actin cytoskeleton that provide the mechanical force for migration and neurite outgrowth [23]. The actin-severing activity of cofilin is exerted through dephosphorylation by SSHs [35,36]. Furthermore, SSH1 dephosphorylates and inactivates LIM kinases (LIMK1 and 2), which in turn contributes to cofilin activation [34]. Thus, 14-3-3

![Fig. 5. Pctaire1 induces process formation and promotes fusion of skeletal muscle cell. C2C12 cells were transfected with the indicated expression vectors. (A) After 1 day of transfection, semi-confluent cells were stained with DAPI (blue). Representative images were displayed in left panel. Ph denotes Phase contrast image. Cell length was then measured in cells expressing the indicated proteins (right graph). Statistical analysis: Dunnett’s multiple comparison test, *P < 0.01 vs WT and §P < 0.01 vs EGFP-V5. Red bars indicate mean values. (B) Differentiated cells were immunostained with an anti-MHC antibody (red) after 3 days of culture in DM. The number of nuclei in cells expressing the indicated proteins (green) was counted by DAPI staining (blue). The fusion index was then calculated as ratio of the number of nuclei in multinucleated cells to the total number of nuclei including mononucleated cells (right graph). Error bars indicate S.D. (N = 3, each N consists of 100 < cells).](image-url)
isoforms can negatively regulate cofilin activity by scaffolding its regulators. Importantly, Pctaire1 is considered to be involved in the activation of cofilin via inactivation of PAK1-LIMK cascade in neuronal cells [23]. Taking these findings into account, we speculate that Pctaire1 competes with SSHs for binding to 14–3–3 proteins, and consequently SSHs can serve as a cofilin activator, which results in the promotion of actin cytoskeletal reorganization required for the process of myogenesis. To validate our hypothesis, we examined cofilin activity by monitoring its phosphorylation state. However, no significant difference was observed in cells overexpressing Pctaire1 or stably expressing shPctaire1, compared with their respective controls (data not shown). This result may suggest that the effect of Pctaire1 on cofilin activity in skeletal muscle cells is no more than that in nervous system.

Contrary to expectations from our cell-based approach, no defect of muscle development has been reported in Pctaire1 knockout mice [17]. As previous studies have suggested that a high degree of functional redundancy among Cdk family members guarantees cell cycle progression in mice lacking Cdk2 and in several Cdns-depleted cells [37–39], other Cdns may complement the function of Pctaire1 in myogenesis. The most likely candidates for the redundant function are Pctaire2/3 (Cdk17/18) in which both domains surrounding Ser119 and Ser153 of Pctaire1 are present. Importantly, these two domains are unique to the Pctaire subgroup of Cdk family [17,18]. Pctaire2/3 are also mainly expressed in brain and/or testis as Pctaire1 [18], however, their functional role in myogenesis remains poorly characterized. Intriguingly, the expression levels of Pctaire2/3 in mouse skeletal muscle is relatively low compared to Pctaire1, though a certain level of Pctaire1–3 transcripts has been observed in C2C12 mouse skeletal muscle myoblasts (BioGPS), suggesting the possibility that function of Pctaire1 during myogenesis is more specialized than that of other Pctaires.

Although further study is required to clarify the precise molecular mechanism by which Pctaire1 promotes the process of myogenesis and to define molecules overlapping the Pctaire1 function, in summary, our findings indicate that increased migration and elongation of cells due to Pctaire1 are important factors in the effective myogenesis.

Acknowledgements

The authors would like to acknowledge Dr. Natsuki Matsushita ( Ehime University Graduate School of Medicine) for kindly providing the CSII-CMV-GFP-RES-Bsd-H1 vector. We thank Dr. Atsuko Sehara (Kyoto University) for kindly providing the C2/4 (C2C12) cells. This work was supported by a Grant-in-Aid for JSPS Fellows (K.S.) and a Grant-in-Aid for Scientific Research (B) (T.S.) from the Ministry of Education, Culture, Sports, Science, and Technology in Japan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.05.060.

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