Specific knockdown of m-calpain blocks myogenesis with cDNA deduced from the corresponding RNAi

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Honda M, Masui F, Kanzawa N, Tsuchiya T, Toyo-oka T. Specific knockdown of m-calpain blocks myogenesis with cDNA
blast to multinucleated myotubes is crucial for myogenesis. Both μ- and m-calpain are ubiquitously expressed in most cells and are
particularly abundant in muscle cells. Knockout of calpain-1 (cat-
ylytic subunit of μ-calpain) induced moderate platelet dysaggre-
gregation, preserving the normal development and growth, although
knockout of calpain-2 (m-calpain) is lethal in mice. Therefore,
there should be muscle-specific function of m-calpain per se.
Previous methods lack direct evidence for the involvement of
m-calpain, because the specific inhibitor to m-calpain has not been
developed yet and the inhibition was less potent. Here, we show
that screened RNA interference (RNAi) specifically blocked the
m-calpain expression by 95% at both the protein and the activity
levels. After transfection of adenovirus vector-mediated cDNA
corresponding to the RNAi-induced short hairpin RNA, m-calpain
in C2C12 myoblasts was knocked down with no compensatory
overexpression of μ-calpain or calpain-3. The specific knockdown
strongly inhibited the fusion to multinucleated myotubes. In addi-
tion, the knockdown modestly blocked ubiquitous effects, includ-
ing cell migration, cell spreading, and alignment of central stress
fiberlike structures. These results may indicate that m-calpain
requiring millimolar Ca2+ level for the full activation plays spe-
cific roles in myogenesis, independent of μ-calpain, and leave us
challenging problems in the future.

RNA interference; muscle cell development; fusion; adenovirus

CALPAINS FORM A SUPERFAMILY of Ca2+-activated cytosolic cy-
tein proteases widely distributed from mammals to inverte-
brates. The conventional calpains (μ- and m-calpain) are com-
posed of heterodimer with each catalytic subunit, encoded by
Capn1 or calpain-2 (Capn2), and a common regul-
atory subunit encoded by calpain-4 (Capn4). Calpain activity
is regulated by a variety of factors, including Ca2+, phospho-
lipids, the small subunit, an endogenous calpain-specific inhib-
itor peptide, calpastatin, autodigestion, and phosphorylation
via the ERK/MAPK pathway (21, 40). Calpain family has been
implicated in a large number of physiological processes, in-
cluding cell spreading, cell migration, myoblast fusion, cell
cycle, and apoptosis (21), and in various pathological pro-
cesses, such as neuromuscular diseases, cardiac dysfunction,
cataract, and diabetes (24, 27, 43, 50). Skeletal and cardiac
muscles contain large amounts of μ- and m-calpain that may
contribute to the progression of muscular dystrophy and/or
advanced heart failure (12, 44, 48), although the lack of a
specific inhibitor for each calpain has made verification of each
role difficult.

Transgenic animals are of great use for uncovering the
physiological function of novel proteins and clarifying the
molecular mechanism of several diseases, developing a new
strategy for treatment. The knockout mice are, however, lim-
ited by the resultant developmental effects, genetic compensa-
tion, and lack of specificity, not at the whole animal level but
at the cellular and/or organ level. In the case of Capn2, the
homeozygous disruption of the gene showed preimplantation
lethality, indicating that this protease is indispensable for early
embryogenesis (16). Here, we used RNA interference (RNAi)
to generate a specific knockdown of Capn2 at the cellular level.
A major challenge in applying this technique in vitro or in vivo
has been addressed by introducing the small interfering RNA
(siRNA) and short hairpin RNA (shRNA) into primary cultures
or into target cells of higher living organisms (18, 29, 47, 49).

We generated Capn2 knockdown of the skeletal myoblast
cell line C2C12, using an efficient adenovirus-mediated RNAi
(37), and demonstrated clear evidence that m-calpain is in-
volved in fusion of myoblasts to myotubes, in addition to other
aspects of myogenesis.

MATERIALS AND METHODS

Materials. Anti-m-calpain antibody was kindly supplied by Dr. H.
Sorimachi, Tokyo Metropolitan Institute for Clinical Sciences. Anti-
α-tubulin (clone DM 1A) and anti-vinculin (clone hVIN1) antibodies
were purchased from Sigma (St. Louis, MO). Alexa Fluor 594-labeled
phalloidin was from Molecular Probes, Invitrogen (Carlsbad, CA). All
other reagents were from Sigma.

Cell culture. C2C12 cells supplied from Riken Gene Bank
(Tsukuba, Japan) were cultured in growth medium (GM), Dulbecco’s
modified Eagle’s medium (DMEM) with 10% fetal bovine serum, as
described previously (36). To promote differentiation from skeletal
myoblasts to myotubes and myocytes, the medium was replaced by the
differentiation medium (DM) containing 2% horse serum after the
cultured cells became confluent in GM.

Virus-mediated gene silencing of Capn2 by RNA interference.
The BLOCK-iT Adeno Expression System (Invitrogen) was used for
creating a replication-incompetent adenovirus that transiently deliv-
ered a shRNA of Capn2 to C2C12 for RNAi. Hairpin RNA was
designed to target specific regions of mouse Capn2 (GenBank acces-

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sion no. NM_009794) mRNA. A control with a scrambled sequence lacked homology to any known Mus musculus mRNAs.

We synthesized two sets of oligonucleotides (Invitrogen): shcapn2 (top, 5'-CACCGGACGAGTTGAAAAATCCGAAGGTTAATTTCTGAGTCTTTCTGCC-3'; bottom, 5'-AAAAGGCAAGATTTCAAGATCTCGCTTGGTATTCTGATTTCTGAGTTGACTGC-3') and shSCR (top, 5'-CACCGCTACAAACAATCGCGATTTCTGAGTTGACTGC-3'; bottom, 5'-AAAACACAATAATCGCGATTTCTGAGTTGACTGC-3').

These oligonucleotides were annealed and cloned into pENTR/U6 vector according to the manufacturer’s instructions. All clones were verified by direct sequencing. The U6 promoter, hairpin sequence, and terminator sequences were ligated into a pAd/BLOCK-it DEST vector. Adenovirus expression plasmids were digested with Pac I to expose the inverted terminal repeats and were transfected into the 293A producer cells with Lipofectamine 2000 (Invitrogen) to produce adenovirus stock. Amplified adenovirus was used to knock down calpain-2, and the enzyme expression was analyzed by Western blot and casein zymography for verification of the expression at the protein and activity levels, respectively.

Quantitative mRNA assay. The quantity of mRNA from cultured cells was measured with a branched DNA signal amplification assay (Quantigene High Volume bDNA Signal Amplification Kit; Panomics, Fremont, CA), following the manufacturer’s instructions. The premises for this assay have been extensively described by Hartley and Klaassen (22).

Western blot analysis. Protein levels of m-calpain large subunit and α-tubulin in C2C12 myoblasts and myotubes were measured as described previously (38, 42). Protein concentrations were determined by Bradford's method (9). After the blotted membrane was washed with Tween 20/PBS, reacted bands were detected using horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG (DAKO, Glostrup, Denmark) with ECL (GE Healthcare Bio-Sciences, Piscataway, NJ).

Calpain activity assay. Both m- and m-calpain activities in cell extracts were simultaneously measured by casein zymography in a nondenaturing system (35).

Immunofluorescence microscopy. C2C12 myoblasts grown on Lab-Tek II chamber slides (Nalge Nunc International, Rochester, NY) were double-stained with Alexa Fluor 594-labeled phalloidin for actin and FITC-labeled specific antibody to vinculin (26, 38). After being washed with PBS, the specimens were examined with a confocal laser scanning microscope (LSM410, Carl Zeiss, Oberkochen, Germany).

Cell motility assay. C2C12 myoblasts were tested for the ability to move into a denuded area on the culture dish (Nalge Nunc International).

Fig. 1. Expressions of calpain-2 mRNA and protein in C2C12. A: mRNA levels of Capn2 at different stages measured with the Quantigene system, as described in MATERIALS AND METHODS. Error bars indicate SEs. *P < 0.05 by Student’s t-test. B: Western blotting of full-length calpain-2 and α-tubulin. C: quantification of full-length calpain-2 protein levels at different stages. Error bars indicate SEs.

Fig. 2. Suppression of calpain-2 expression at protein and activity levels with adenovirus vector-mediated RNAi in C2C12. A: Western blotting of calpain-2 and α-tubulin at 3 days after transfection with Ad_shSCR (nonsilencing control; SCR) or Ad_shcapn2 (targeting calpain-2; capn2). B: zymography of 3-day posttransfection cells. C: sustained inhibition of calpain-2 from 5 to 9 days after transfection, analyzed with Western blotting (top) or zymography (bottom). D: comparison of relative activity levels of µ- and m-calpain in Capn2 knockdown cells. The activities at days 5, 7, and 9 were compared with the activity at day 3. Error bars indicate SEs. *P < 0.05 and ***P < 0.001 by Student’s t-test.
Phase contrast pictures were taken at 0 and 24 h, and the cell migration was determined by the distance moved into the acellular area over time.

**Spreading assay.** Cell morphology was examined by fluorescent microscopy and optical microscopy, and the number of cells presenting visible cytoplasm or not was determined by visual inspection on Lab-Tek II chamber slides. The rate of spreading was defined as the number of cells with visible cytoplasm/total number of cells.

**Statistical analysis.** For the quantitative assay, the differences between the Ad_shSCR- and Ad_shcapn2-transfected cells were evaluated by Student’s t-test. $P < 0.05$ was considered significant.

Fig. 3. Facilitation of cell detachment by induction of differentiation in Capn2 knockdown cells. Ad_shSCR (A) and Ad_shcapn2 (B) were transfected. The C2C12 cell culture was continued in DMEM containing 10% fetal bovine serum for 3 days up to the confluency. The medium was then replaced by DMEM containing 2% horse serum, and cell differentiation was induced after 3 days. Bar, 150 μm. C: relative number of detachment cells per 4 mm$^2$. Error bars indicate SEs. ***$P < 0.001$ by Student’s t-test.

Fig. 4. Inhibition of multinucleated myotubes formation in adenovirus vector-mediated Capn2 knockdown cells. Three days after transfection, C2C12 myoblasts were reinfected with Ad_shSCR (A) or Ad_shcapn2 (B), and the medium was replaced by differentiation medium. On day 7, these cells were examined with light microscopy. Bar, 150 μm. C: the numbers of myotubes in Ad_shSCR and Ad_shcapn2 were counted in 4 mm$^2$. A myotube was defined as a cell showing at least three nuclei. Error bars indicate SEs. ***$P < 0.001$ by Student’s t-test. D: suppression of Capn2 on day 7 after retransfection, analyzed with Western blotting (top) or zymography (bottom).

Fig. 5. Reduced migration of myoblasts after adenovirus vector-mediated Capn2 knockdown. C2C12 cells were transfected with Ad_shSCR or Ad_shcapn2 for 3 days. After 24 h in a quiescent medium, confluent myoblasts were scraped off with a pipette tip, and medium was replaced. The numbers of myoblasts that migrated into the wound site were counted under microscopy. The data represent the average of multiple fields per experiment from 5 separate experiments. Error bar indicates SE. **$P < 0.01$ by Student’s t-test.
RESULTS

Expressions of m-calpain large subunit (calpain-2) mRNA and protein during C2C12 myogenesis. To determine the expression level of calpain-2 at different stages of myogenesis, we quantified it at both mRNA and protein levels in mouse C2C12. Cells were extracted at the subconfluency (day - 1) and confluency (day 0) from the GM cultures and at various stages after the induction of cell differentiation (days 3-18) from DM cultures. Although expression levels of Capn2 mRNA fluctuated slightly at different stages (P = 0.02-0.04; Fig. 1A), the protein level of full-length calpain-2 showed no significant difference at these stages (P > 0.05; Fig. 1, B and C). No clear correlation was detected between amount of the transcript and the transgene, so the level of calpain-2 protein may be under the influence of posttranslational modifications, folding of the expressed polypeptide, or half-life of the mRNA. We conclude that the protein level of full-length calpain-2 was stable and constitutively expressed in both proliferating and differentiating myoblasts.

Suppression of calpain-2 by adenovirus vector-mediated RNAi in C2C12 myogenesis. It was difficult to effectively transfect synthetic siRNA or siRNA-expressing plasmids in myoblasts, myotubes, and myocytes. For the complete expression of siRNA to Capn2 in C2C12 myoblasts, the adenovirus vector was very useful, because the transfection efficiency reached nearly 100% (4). The adenovirus-mediated RNAi was generated by expressing U6 promoter-driven shRNA (Ad_shcapn2), which targets Capn2, as well as the control vector with a scrambled sequence (Ad_shSCR). At 3 days after the transfection, C2C12 cells expressing Capn2-RNAi showed an apparent reduction of calpain-2 protein level (Fig. 2A). To simultaneously assess both activities of μ- and m-calpain in the knockdown cells at 3 days after transfection, casein zymography was carried out. Both enzyme activities were observed in Ad_shSCR-transfected cells, but the knockdown showed only μ-calpain activity with no m-calpain activity (Fig. 2B).

Additionally, to assess the continuous reduction of RNAi-mediated calpain-2, we followed the time course of calpain-2 level up to day 9 after transfection and found that the expressed amount gradually recovered in the posttransfection period (Fig. 2C). This reversal may reflect a transient action after the target gene delivery. However, these data demonstrate the potential to suppress calpain-2 with Ad_shcapn2. The activity of calpain-3

Fig. 7. Reduced spreading of Capn2 knockdown cells. At 3 days after transfection with Ad_shSCR or Ad_shcapn2, C2C12 myoblasts were plated on the noncoated chamber slides for 3 h. These cells were stained with vinculin antibodies and Alexa Fluor 594-labeled phalloidin as described in MATERIALS AND METHODS. Rates of spreading were measured by determining the ratio of numbers of spreading cells to numbers of total cells. Error bars indicate SEs. **P < 0.01 by Student’s t-test.
specifically contained in skeletal muscle (30) was not detected at all in the current zymography. It should be noted that no compensatory expression of \( \mu \)-calpain large subunit (calpain-1) was detected during the suppression of calpain-2. The \( \mu \)-calpain activity of cells transfected with Ad_shcapn2 decreased to 5–27\% (\( P < 0.0005 \)), compared with that with Ad_shSCR on days 3 to 7 after the transfection (Fig. 2, B and C). However, at that time, no significant difference was observed in the activity of \( \mu \)-calpain between Ad_shSCR and Ad_shcapn2 (\( P > 0.05 \); Fig. 2D). On day 9 after the transfection, the knockdown efficiency recovered up to 40\% and the activity of \( \mu \)-calpain slightly increased, compared with Ad_shSCR (\( P = 0.045 \); Fig. 2D).

Cell detachment during the differentiation of Capn2 knockdown. Myoblasts were at first grown in GM and then induced to differentiate by switching to DM. The alignment of myoblasts started from days 3 to 4, followed by the fusion to multinucleated myotubes between days 5 and 7. Previous reports postulated that m-calpain was essential for myoblast differentiation to myotubes via the limited digestion of membrane proteins (25). We examined whether knockdown of Capn2 inhibits the myoblast fusion and/or differentiation to myotubes. On day 3 after the transfection when these cells reached the confluence, we started the differentiation. Myoblasts transfected with Ad_shSCR became aligned and started to fuse on day 3 after the induction of differentiation. However, those cells transfected with Ad_shcapn2 did not fuse (Fig. 3, A and B). Furthermore, Capn2 knockdown cells had changed morphology and diminished adhesiveness, resulting in numerous detachments from the dish (Fig. 3C).

Inhibition of myoblast fusion to multinucleated myotubes with the selective knockdown of Capn2. Because the duration of adenovirus-vector mediated expression of both the transcript and the transgene is transient, the permanent knockdown is not expected. Actually, the knockdown was restored from day 7 after the transfection (Fig. 2C). For an exact assessment of the inhibitory effect of RNAi, it is necessary for evaluating myoblast differentiation to keep the high knockdown activity. We...
repeated the transfection on day 3 after the initial treatment. Then, we counted the number of myotubes (a myotube was defined as a cell with at least three nuclei).

In control cells on day 7 in DM, fusion to multinucleated myotubes/myocytes was observed after successive transfection on day 3 with Ad_shSCR (Fig. 4, A and C). In contrast, the Capn2 knockdown cells showed neither fusion nor differentiation to mature myotubes or myocytes (Fig. 4, B and C). In addition, there were fewer nuclei and smaller myotubes in Ad_shcapn2-transfected cell cultures compared with the control (Fig. 4, A and B). Retransfection of the adenovirus vector on day 3 after the initial transfection prolonged the RNAi action up to day 7 while maintaining the constant expression of m-calpain (Fig. 4D). Thus, we conclude that the Ad_shcapn2 has strongly inhibited the myoblast fusion and the inhibition was independent of µ-calpain.

Reversed migration and altered morphology after Capn2 knockdown. Calpain-deficient embryonic fibroblasts have been reported not to regulate the membrane protrusion dynamics during fibroblast motility (19, 20). To evaluate whether the specific knockdown of Capn2 affects skeletal myoblast migration, we examined cell motility at 3 days after transfection with Ad_shSCR or Ad_shcapn2. Cell movement was analyzed by wound healing assay. An area of a monolayer culture was denuded, and the number of cells that traveled toward the acellular front was measured. Neither protein nor activity levels of m-calpain were observed in the C2C12 cells transfected with Ad_shcapn2 for 3 days (data not shown). These cells showed distinctly reduced migration rates, compared with control cells transfected with Ad_shSCR (Fig. 5), providing evidence that m-calpain makes a significant contribution to cell motility. Morphologically, these knockdown cells transfected with Ad_shcapn2 for 3 days revealed numerous membrane protrusions and filopodia at 1 h after the plating (Fig. 6, A and B) and maintained the structure up to day 4 after the transfection (Fig. 6, C and D).

Disruption of architecture of cytoskeleton during the myoblast spreading. Functional assessment of m-calpain was applied to the myoblast spreading. The C2C12 cells transfected with Ad_shSCR or Ad_shcapn2 for 3 days were plated on the noncoated chamber slides and monitored from 10 min to 3 h. In the control Ad_shSCR-transfected cells, the number of spreading cells gradually increased. In contrast, the cell spreading was delayed in the Ad_shcapn2-transfected cells. A large number of cells kept the round morphology for 3 h. At 3 h after the plating, 80.5 ± 3.9% cells had spread in the control slides. However, Capn2 knockdown cells showed a reduced spreading rate (64.3 ± 7.1%; P < 0.01, Fig. 7). These results indicated that the defect in spreading was related to the inhibition of m-calpain activity.

Furthermore, the distribution of cytoskeleton in Capn2 knockdown cells differed from that of the control cells. To explore whether the knockdown of Capn2 affects the cytoskeletal organization, we plated myoblasts transfected with Ad_shSCR or Ad_shcapn2 for 5 days on the chamber slides and observed the cytoskeleton using double-fluorescence microscopy. Actin fibers were visualized with Alexa Fluor 594-labeled phalloidin. Vinculin reported to be hydrolyzed with m-calpain (21) was detected with the specific antibody labeled with FITC (Fig. 8). Ad_shSCR-transfected cells contained numerous stress fiberlike structures (SFLSs) with focal adhesions. However, Capn2 knockdown cells lost SFLSs, particularly the central SFLSs (Fig. 8D). We also observed ruffled membranes in the Ad_shcapn2-transfected cells and a loss of vinculin containing focal adhesions at the cell periphery. These findings indicate that m-calpain plays an important role in regulating the localization of actin cytoskeleton and focal adhesion.

**DISCUSSION**

In the present study, we have generated an in vitro knockdown system for m-calpain to evaluate the physiological effects of decreased m-calpain activity, including muscle-specific differentiation per se from myoblasts to myotubes/myocytes and the general mechanism of cell locomotion via cytoskeletal organization. For the first time, we have demonstrated the following four main results: 1) selective loss of m-calpain enzyme and, accordingly, its activity; 2) the strong inhibition of m-calpain with no direct effect on µ-calpain activity; 3) the ceasing of myoblast development to myotubes and/or myocytes; and 4) a partial blocking of the locomotion...
and proliferation (Fig. S1; the online version of this article contains supplemental data) of myoblasts.

In a wide variety of cells such as fibroblasts, myoblasts, endothelial cells, and cancer cells (1, 2, 6, 7, 15, 16, 19, 23, 28, 34, 46), calpains have been implicated in many aspects of cell physiology, including the cell spreading, migration, and actin remodeling (Table 1). However, the absence of fully specific calpain inhibitors has so far prevented unambiguous proof of a particular role. Previous methods (6, 7, 13, 14, 28) were insufficient for both qualitative and quantitative purposes, i.e., less specific for discriminating each calpain isofrom and not completely suppressing the target calpain in a pinpoint manner. Thus, the RNAi strategy, which can inhibit each calpain specifically, would be a powerful tool to clarify physiological functions.

Despite stable expressions of µ- and m-calpain (Figs. 1 and 2), both activities would be increased during the myoblast fusion, concomitantly with myotube formation, and restored after the fusion (8, 11). Balances between µ-calpain and its specific inhibitor, calpastatin, or between m-calpain and calpastatin are assumed to determine their net proteolytic activities, when the proteases and inhibitors are freely accessible to one another. The temporary diminution in calpastatin allows the activation of calpain and calpain-induced proteolysis, which is required for myoblast fusion (5). In addition, other mechanisms such as the posttranslational modification (39), dissociation, and/or translocation from the counterpart (21) may be working for enhancement of the enzyme activity. Furthermore, the expression of µ-calpain was independent of m-calpain, suggesting no cross talk between these isofroms. In the present study, the increase of µ-calpain activity was seen in Capn2 knockdown cells, which recovered m-calpain activity up to 40% at 9 days after transfection (Fig. 2D). However, no compensation of µ-calpain activity was detectable at 3 days after transfection (Fig. 2B) and at 7 days after transfection (Fig. 4D) in Capn2 knockdown cells. These data suggest that the expression of µ-calpain is not linked to m-calpain activity. The activation of m-calpain but not µ-calpain is required for induction of the limited proteolysis of membrane proteins that may be closely related to the myoblast fusion. Overall, m-calpain is essential for muscle cell differentiation, especially during the burst of myoblast fusion at the initial stage of differentiation. On the other hand, previous investigations demonstrated that µ-calpain did not affect myoblast fusion (3). Thus, these two isozymes might have distinctly different functions.

Interestingly, the phenomena such as fusion or differentiation to mature myotubes were not seen in filamin C (FLNc) knockout myoblasts as well as Capn2 knockout myoblasts. FLNc is the muscle-specific member of a family of actin binding proteins. The FLNc knockout myoblasts display defects in differentiation and fusion ability and ultimately form multinucleated “myoballs” (10). These data indicate that FLNc is critical for normal myogenesis as well as for the maintenance of the structural integrity of the muscle fibers. Although the causal relation of two similar phenomena is not clear, a number of molecules have been implicated in muscle cell differentiation.

Most studies so far lacked a direct proof that m-calpain, but not µ-calpain, is actually working in myogenesis. Considering intracellular physiological Ca2+ concentration at submicromolar level (17), m-calpain that requires millimolar Ca2+ concentration for the full activation leaves us an exciting challenge in muscle biology. Although treatment by several nonspecific calpain inhibitors has been reported to suppress the progression of muscle diseases (14), the responsible calpain has not been identified. m-Calpain plays an indispensable role in murine embryogenesis, possibly related to preimplantation development (16).

In fibroblasts of the Capn4−/− mouse that has lost both µ- and m-calpain, similar morphological change in Capn2 knockdown was observed, showing numerous protrusions (15, 19). These Capn2 knockdown cells had only µ-calpain activity (data not shown). Protrusion may reflect the polymerization of actin filaments at the barbed ends and their formation of a highly branched dendritic network that drives membrane extension at the leading edge of lamellipodia (32). Huttenlocher’s group has indicated that the membrane protrusion is regulated by m-calpain-mediated proteolysis of cortactin in vivo (31, 32). Additionally, cortactin may play a key role in the dynamic
assembly and disassembly in actin polymerization at the cell periphery (45). Cleavage of other cytoskeletal proteins, such as talin, spectrin, and focal adhesion kinase, has been considered to be responsible for abnormal organization of cytoskeleton. The findings of the present study that knockdown of Capn2 lost SFLSs strongly suggests the involvement of m-calpain among calpain family members in the formation of SFLSs in the myoblasts (33). Integrin-mediated motility decreased in Capn4−/− fibroblasts that lack both μ- and m-calpain (15), and calpain inhibition may negatively modulate cell migration through the inhibition of new adhesions and the destabilization of the cytoskeleton (13). We have observed similarly reduced migration in Capn2 knockdown cells. Recent investigation demonstrated that channel kinase transient receptor potential melastatin 7 localizes to peripheral adhesion complexes with m-calpain, where it regulates cell adhesion by controlling the protease activity (41). Cell adhesion is regulated through m-calpain by mediating the calcium influx into peripheral adhesion complexes. Thus, m-calpain would play dual roles: 1) regulation of migration of various kinds of cells and 2) muscle-specific fusion during differentiation. These functions may be closely related to an invasion or metastasis of cancer cells and to the development of muscle or cardiac diseases in clinical settings, leaving us fascinating problems to be resolved in both basic and clinical sciences.

GRANTS

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