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Hypertrophy and atrophy inversely regulate Caveolin-3 expression in myoblasts

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Abstract

Caveolin-3 (Cav-3) is a muscle-specific membrane protein crucial for myoblast differentiation, as loss of the protein due to mutations within the gene causes an autosomal dominant form of limb girdle muscular dystrophy 1-c. Here we show that along with p38 activity the PI3-kinase/AKT/mTOR pathway is required for proper Cav-3 up-regulation during muscle differentiation and hypertrophy, as confirmed by the marked increase of Cav-3 expression in hypertrophied C2C12 cells transfected with an activated form of AKT. Accordingly, Cav-3 expression was further increased during hypertrophy of L6C5 myoblasts treated with Arg^8-vasopressin and in hypertrophic muscles of MLC/mIGF-1 transgenic mice. In contrast, Cav-3 expression was down-regulated in C2C12 myotubes exposed to atrophic stimuli such as starvation or treatment with dexamethasone. This study clearly suggests that Cav-3 expression is causally linked to the maturation of muscle phenotype and it is tightly regulated by hypertrophic and atrophic stimuli.

Keywords: Caveolin 3; Hypertrophy; AKT; Igf-1; Vasopressin; Atrophy; Starvation; Dexamethasone

Mammalian caveolins consist of three integral membrane proteins, termed Cav-1, Cav-2, and Cav-3 [1–4], that represent the main structural elements of caveolae, 50–100 nm invaginations of the plasma membrane [5–7]. Caveolins, acting as scaffolding proteins, are able to concentrate lipids (cholesterol and glycosphingolipids [8–10]), signalling proteins (G-proteins, H-Ras, nitric oxide synthase, Src-like kinases [8,11–16]), and structural proteins (dystroglycan, M-phosphofructokinase and dysferlin [17–20]) in the caveolae.

Caveolins 1 and 2 have a similar tissue distribution, and form hetero-oligomeric complex in different cell types, as adipocytes, endothelial cells, pneumocytes, and fibroblasts [21], whereas Cav-3 expression is restricted to skeletal muscle and heart [18]. During myotube formation Cav-3 expression is strongly up-regulated by activation of p38 pathway [22], resulting localized at the plasma membrane of muscle fibers (sarcolemma) in complex with dystrophin and its associated glycoproteins [18]. The crucial role of Cav-3 in muscle was clearly demonstrated by the finding that mutations in the Cav-3 gene are responsible for an autosomal dominant form of limb girdle muscular dystrophy 1-c [23]. These mutations cause the loss of Cav-3 protein, as the mutated Cav-3 proteins associated with the endogenous wild type protein undergo proteosomal degradation [24]. Interestingly, the overexpression of Cav-3 in muscle mice results in a Duchenne-like phenotype [25], clearly demonstrating that a tight regulation of Cav-3 expression is crucial for proper myofiber organization.

In this study we demonstrate that the activation of both p38 and PI3-kinase/Akt/mTOR pathways is required for proper Cav-3 up-regulation during muscle differentiation.

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; HS, horse serum; PBS, phosphate buffer solution; BSA, bovine serum albumin.

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Subsequently, we analyzed the expression pattern of Cav-3 in both muscle hypertrophy and atrophy. Our result suggest that Cav-3 is inversely regulated during these processes, as in vitro and in vivo hypertrophy leads to increased Cav-3 expression, whereas myotube atrophy impairs Cav-3 expression.

Materials and methods

Cell culture and pharmacological treatments. The mouse C2C12 myoblasts were maintained at subconfluent density at 37 °C in 5% CO_2 and cultured in DMEM high glucose (Sigma–Aldrich) supplemented with 10% FBS (Sigma–Aldrich) and 100 μg/ml penicillin–streptomycin antibiotic (Sigma–Aldrich), defined as growth medium (GM). Confluent cells were shifted to differentiation medium (DM) containing DMEM supplemented with 2% HS and the medium was changed every two days. Pharmacological treatments of myoblasts were performed every day using 10 μM SB239063 (Sigma–Aldrich) to inhibit p38 activity, 10 μM LY294002 (Sigma–Aldrich) to inhibit PI3-kinase activity, 5 ng/ml rapamycin (Sigma–Aldrich) to inhibit mTOR activity. The rat L6C5 myoblasts were seeded at 25,000/cm^2 in GM, and twenty-four hours after plating the cultures were shifted to serum-free medium consisting of DMEM supplemented with 1% fatty acid-free BSA (Sigma–Aldrich) and treated with synthetic 0.1 μM Arg^8-vasopressin (AVP, Sigma–Aldrich) for different time-points.

To induce myotube atrophy, C2C12 cells grown in DM were either the serum, glucose, and aminoacid starved or added with 100 μM dexamethasone (Sigma–Aldrich) at the indicated different time-points.

Cell staining and myotube quantification. To visualize myotubular structures, cells were washed three times in PBS before fixing for 10 min in 100% methanol at −20 °C. Cells were stained with Giemsa reactive (Sigma–Aldrich) for 2–3 h and again washed in PBS. To quantify the myotube diameter, 10 fields were chosen randomly and 10 myotubes were measured per field. The average diameter per myotube was the mean of 10 measurements taken along the length of the myotube.

Stable transfections. To obtain C2C12 myoblasts expressing the constitutive active form of AKT, the cells were transfected using a pBABE vector in which a myristoylated AKT was cloned. After transfection by Lipofectamine 2000 reagent, the mix of stable transfectants were obtained after 10 days selection in puromycin antibiotic (2 μg/ml, Sigma–Aldrich) and used by few passages.

Western blot analysis. Protein concentration was obtained by bicinchoninic acid assay (Pierce). For Cav-3 immunoblotting, myoblast cells were washed twice with PBS and lysed 30 min at 4 °C in a buffer containing 10 mM Tris, pH 8.0, 0.15 M NaCl, 5 mM EDTA, and 1% Triton X-100 added with a mix of protease inhibitors (Roche Molecular Biochemicals). Samples were centrifuged at 14,000 rpm for 10 min at 4 °C. Pellet (insoluble fraction) was resolved by SDS–PAGE (12% acrylamide) and a mouse monoclonal antibody against Cav-3 was used (clone 26, BD Transduction Laboratories). Muscle tissues from wild type and transgenic MLC/mIgf-1 mice [26] were homogenized in presence of liquid nitrogen and sonicated in a buffer containing 10 mM Tris (pH 8.0), 0.15 M NaCl, 5 mM EDTA, 1% Triton X-100 and 60 mM octyl-glucoside added with a mix of protease inhibitors. In addition, samples were centrifuged at 13,000*g* for 10 min at 4 °C to remove insoluble proteins. Soluble proteins were then resolved by SDS–PAGE (12% acrylamide) and transferred to nitrocellulose membranes before Cav-3 detection. For the detection of GATA-2 and the phosporylated forms of AKT and P70S6 K, myoblast cells were harvested at 4 °C in RIPA lysis buffer (1% Nonidet P40, 0.5% sodium deoxycholate, 0.1% SDS in 50 mM NaCl, 20 mM Tris–HCl pH 7.6) containing a mix of protease inhibitors. Lysates were cleared by centrifugation at 12,000*g* for 15 min at 4 °C before resolving the surnatants by SDS–PAGE (10% acrylamide). GATA2 was detected using a mouse monoclonal antibody (clone CG2-96, Santa Cruz Biotechnology). The phosphorylated forms of AKT (Ser^{473}) and P70S6 K (Thr^{389}) were detected using rabbit polyclonal antibodies (Cell Signalling). An antibody against alpha tubulin (Sigma–Aldrich) was used to normalize the loading. Western blots were revealed with enhanced chemiluminescence (Chemicon).

Statistics. All of the data are expressed as means ± SE Statistical significance was determined using *t*-Student's analysis. A p value of <0.05 was considered significant.

Results and discussion

PI3-kinase/AKT/mTOR and p38 pathways are both required for Cav-3 expression during myoblast differentiation

Cav-3 has long been known to be required for proper myoblast differentiation, as its targeted down-regulation is sufficient to inhibit myotube formation in C2C12 cells [18,22]. It has been previously reported that activation of p38 pathway is required to up-regulate Cav-3 expression during myotube formation [22]. In this study, we further explored the effects of PI3-kinase/AKT/mTOR pathway inhibition on Cav-3 expression, being the activation of this pathway crucial for C2C12 myoblast differentiation [27,28]. As expected, the inhibition of either p38 activity or PI3-kinase/AKT/mTOR pathway by pharmacological treatments impaired myotube formation of C2C12 cells (data not shown). As shown in Fig. 1, terminally differentiated C2C12 cells, cultured for 4 days in differentiating medium (DM), exhibited up-regulation of Cav-3 expression. In contrast, the pharmacological inhibition of each p38, PI-3 kinase and mTOR activity by addition of either 10 μM SB239063, 10 μM LY294002 or 5 ng/ml rapamycin delayed and significantly decreased Cav-3 expression. These data suggest that the activation of p38 and PI3-kinase/AKT/mTOR pathways are both necessary to determine the physiological Cav-3 up-regulation during the stages of myotube formation.

In vitro and in vivo myotube hypertrophy enhances Cav-3 expression

Skeletal muscle hypertrophy plays an important role during post-natal development occurring in response to muscle overload and resulting in an increase of transla-

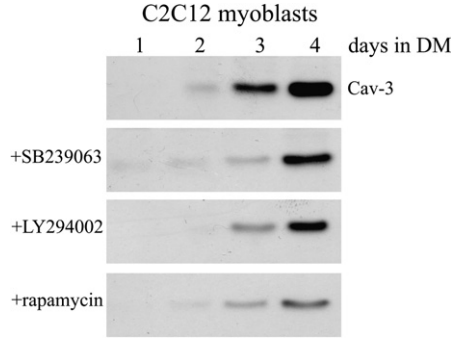

Fig. 1. Inhibition of both p38 and PI3-kinase/AKT/mTOR pathways down-regulates Cav-3 expression. C2C12 myoblasts were differentiated for 4 days in presence of the indicated treatments and subjected to Western blot analysis of Cav-3 expression using a monoclonal antibody.

tional activity accompanied by increase in fiber size [29,30]. As the sustained AKT activation has been described to induce hypertrophy in myoblasts and mice muscles [30], we analysed whether Cav-3 expression could be further increased during myofiber hypertrophy. To this purpose, C2C12 myoblasts stably transfected with a constitutively activated form of AKT (caAKT cells) were allowed to differentiate until day 5 and then stained to visualize myotubular structures. As shown in Fig. 2A, caAKT myoblasts displayed hypertrophic phenotype compared to parental C2C12 cells, as confirmed by myotube diameter quantification (Fig. 2B, upper panel) and increased phosphorylation of AKT and p70S6 K (Fig. 2B, bottom panel). Interestingly, Cav-3 expression was further increased in hypertrophied caAKT myotubes compared to normally differentiated C2C12 myotubes (Fig. 2C), suggesting that a sustained activation of AKT/mTOR/p70S6 K signalling enhances Cav-3 expression.

In addition, to further prove the activation of Cav-3 expression in mature and hypertrophic myotubes, we used another experimental model, the L6C5 myoblasts, which undergo hypertrophy in response to Arg^8-vasopressin (AVP) [31], a neurohypophyseal nonapeptide constituting a novel family of positive regulators of terminal differentiation [32–34]. As shown in Fig. 2D, AVP treated myoblasts formed larger myotubes compared to control cells and showed a characteristic organization of the nuclei, forming nuclear rings, which represent a morphological marker of muscle hypertrophy and maturation in vitro [35]. Western blot analysis (Fig. 2E), performed over a 5-days time course, showed a significant increase of Cav-3 expression in AVP treated cells compared to normally differentiated

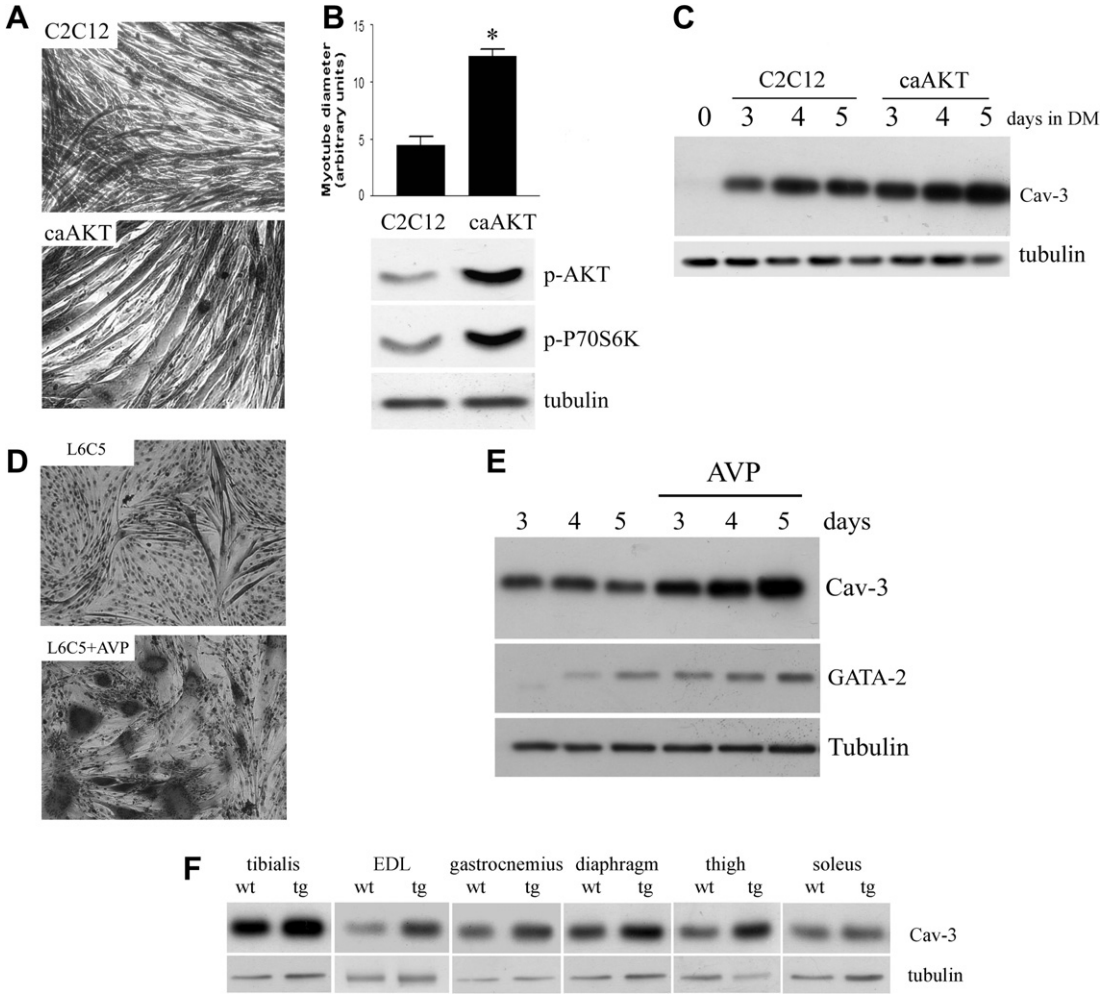

Fig. 2. Myotube hypertrophy enhances Cav-3 expression. (A) The myotubes of C2C12 and caAKT cells grown in DM for 96 h were visualized by Giemsa staining. (B, upper panel) Mean myotube diameters represented in the graph are expressed in arbitrary units ($n = 10$, $^*P < 0.05$). (B, bottom panel) Immunoblot analysis against phospho AKT (Ser^{473}) and phospho P70S6K (Thr^{389}) in parental and caAKT cells grown for 48 h in DM. The data were normalized using tubulin as control. (C) Immunoblot analysis against Cav-3 in parental and caAKT cells performed over a 5 days time course of differentiation. The data were normalized using tubulin as control. (D) Myotube visualization by Giemsa staining of differentiated and AVP-treated L6C5 cells. (E) Immunoblot analysis against Cav-3 and GATA-2 expression in differentiated and AVP-treated L6C5 cells performed over a 5 days time course. The data were normalized using tubulin as control. (F) Immunoblot analysis of Cav-3 expression in muscles from wild-type (wt) and transgenic MLC/mIgf-1 mice (tg). The data were normalized using tubulin as control.

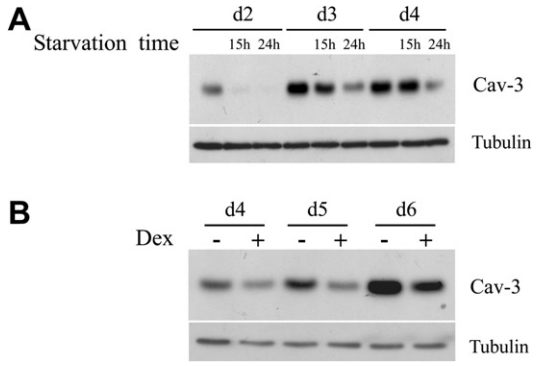

Fig. 3. Myotube atrophy down-regulates Cav-3 expression. (A) Cav-3 expression was detected by Immunoblot analysis in C2C12 myotubes differentiated until day two (d2), three (d3) and four (d4) and starved for 15 and 24 h. The data were normalized using tubulin as control. (B) Immunoblot analysis of Cav-3 expression in d4, d5, and d6 myotubes treated 48 h before with 100 μM dexamethasone (Dex). The data were normalized using tubulin as control.

cells, accompanied by up-regulation of GATA2, a transcription factor which represent a marker of hypertrophy [36].

To definitely correlate Cav-3 activation with muscle hypertrophy, we then investigated Cav-3 expression in hypertrophied muscles from 3-months old transgenic mice expressing a locally active form of Igf-1 under the control of the muscle-specific myosin light chain promoter (MLC/mIgf-1 mice) [26]. As shown in Fig. 2F, Cav-3 expression was increased in transgenic mice compared to the wild-type counterparts in all tested muscles enriched in fast-twitch fibers (tibialis, extensor digitorum longus (EDL), gastrocnemius, diaphragm and thigh), where the MLC regulatory cassette is expressed at high level [26]. Of note, Cav-3 expression remained unalterated in slow muscle such as soleus, where the MLC regulatory cassette is expressed at very low levels [26,37], suggesting that the Cav-3 increase is strictly related to the pathways activated by Igf-1 transgene expression.

Taken together, these data suggest that myofiber hypertrophy obtained by different stimuli such as Igf-1 and AVP is accompanied by Cav-3 up-regulation. Interestingly, as Cav-3 overexpression in transgenic mice resulted in a Duchenne-like phenotype [25], characterized by the presence of necrotic, regenerating and hypertrophic fibers, the Cav-3 increase observed during physiological muscle hypertrophy must be necessarily accompanied by a tight regulation of differentially coordinated events, to avoid muscle degeneration.

Myotube atrophy causes Cav-3 down-regulation

Myotube atrophy is a common response to fasting, disuse and a variety of diseases (e.g., cancer and diabetes mellitus). Under these different conditions, the myofibers undergo increased protein breakdown accompanied by reduced fiber size, primarily due to activation of the ubiquitin–proteasome pathway [38,39]. To investigate whether Cav-3 expression was modulated in atrophied myofibers, we subjected C2C12 myotubes to either starvation (Fig. 3A) or treatment with dexamethasone (Fig. 3B). C2C12 myotubes, differentiated until day two (d2), three (d3) and four (d4) were subjected to starvation for 15 and 24 h, displaying reduced myofiber size (data not shown). Under these conditions, Cav-3 expression resulted significantly down-regulated, with the strongest effect at level of nascent myotubes. Accordingly, the treatment of differentiated myotubes (d4, d5, and d6) with 100 μM dexamethasone for 48 h produced myofiber atrophy accompanied by evident Cav-3 down-regulation. Indeed, it remains to establish whether Cav-3 down-regulation during atrophy is dependent on protein degradation instead of diminished transcript and protein synthesis. Interestingly, the mutated forms of Cav-3 (P104L and ΔTFT) involved in the autosomal dominant form of limb girdle muscular dystrophy 1-c undergo ubiquitination and proteasomal degradation [24], suggesting the presence of ubiquitination sites in the primary Cav-3 sequence, and indicating that also the wild-type Cav-3 might be processed by increased activity of muscle ubiquitin ligases during myotube atrophy.

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References

[1] J.R. Glenney, D. Soppet, Sequence and expression of caveolin, a protein component of caveolae plasma membrane domains phosphorylated on tyrosine in RSV-transformed fibroblasts, Proc. Natl. Acad. Sci. USA 89 (1992) 10517–10521.
[2] P.E. Scherer, T. Okamoto, M. Chun, I. Nishimoto, H.F. Lodish, M.P. Lisanti, Identification, sequence and expression of caveolin-2 defines a caveolin gene family, Proc. Natl. Acad. Sci. USA 93 (1996) 131–135.
[3] Z.L. Tang, P.E. Scherer, T. Okamoto, K. Song, C. Chu, D.S. Kohtz, I. Nishimoto, H.F. Lodish, M.P. Lisanti, Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle, J. Biol. Chem. 271 (1996) 2255–2261.
[4] M. Way, R. Parton, M-caveolin, a muscle-specific caveolin-related protein, FEBS Lett. 376 (1995) 108–112.
[5] M.P. Lisanti, P. Scherer, Z.L. Tang, M. Sargiacomo, Caveolae, caveolin, and caveolin-rich membrane domains: a signalling hypothesis, Trends Cell. Biol. 4 (1994) 231–235.
[6] E.J. Smart, G.A. Graf, M.A. McNiven, W.C. Sessa, J.A. Engelman, P.E. Scherer, T. Okamoto, M.P. Lisanti, Caveolins, liquid-ordered domains, and signal transduction, Mol. Cell. Biol. 19 (1999) 7289–7304.
[7] F. Galbiati, B. Razani, M.P. Lisanti, Emerging themes in lipid rafts and caveolae, Cell 106 (2001) 403–411.

[8] S. Li, K.S. Song, M.P. Lisanti, Expression and characterization of recombinant caveolin, J. Biol. Chem. 271 (1996) 568–573.
[9] M. Murata, J. Peranen, R. Schreiner, F. Weiland, T. Kurzchalia, K. Simons, VIP21/Caveolin is a cholesterol-binding protein, Proc. Natl. Acad. Sci. USA 92 (1995) 10339–10343.
[10] A.M. Fra, M. Masserini, P. Palestini, S. Sonnino, K. Simons, A photo-reactive derivative of ganglioside GM1 specifically cross-links VIP21-caveolin on the cell surface, FEBS Lett. 375 (1995) 11–14.
[11] S. Li, T. Okamoto, M. Chun, M. Sargiacomo, J.E. Casanova, S.H. Hansen, I. Nishimoto, M.P. Lisanti, Evidence for a regulated interaction between heterotrimeric G proteins and caveolin, J. Biol. Chem. 270 (1995) 15693–15701.
[12] K.S. Song, S. Li, T. Okamoto, L. Quilliam, M. Sargiacomo, M.P. Lisanti, Co-purification and direct interaction of Ras with caveolin, an integral membrane protein of caveolae microdomains, J. Biol. Chem. 271 (1996) 9690–9697.
[13] S. Li, J. Couet, M.P. Lisanti, Src tyrosine kinases, Ga subunits, and H-Ras share a common membrane-anchored scaffolding protein, caveolin, J. Biol. Chem. 271 (1996) 29182–29190.
[14] P.W. Shaul, E.J. Smart, L.J. Robinson, Z. German, I.S. Yuhanna, Y. Ying, R.G.W. Anderson, T. Michel, Acylation targets endothelial nitric-oxide synthase to plasmalemmal caveolae, J. Biol. Chem. 271 (1996) 6518–6522.
[15] G. Garcia-Cardena, P. Oh, J. Liu, J.E. Schnitzer, W.C. Sessa, Targeting of nitric oxide synthase to endothelial cell caveolae via palmitoylation: Implications for nitric oxide signaling, Proc. Natl. Acad. Sci. USA 93 (1996) 6448–6453.
[16] G.M. Smythe, J.C. Eby, M.H. Disatnik, T.A. Rando, A caveolin-3 mutant that causes limb girdle muscular dystrophy type 1C disrupts Src localization and activity and induces apoptosis in skeletal myotubes, J.Cell Sci. 116 (2003) 4739–4749.
[17] F. Sotgia, J.K. Lee, K. Das, M. Bedford, T.C. Petrucci, P. Macioce, M. Sargiacomo, F.D. Bricarelli, C. Minetti, M. Sudol, M.P. Lisanti, Caveolin-3 directly interacts with the C terminal tail of beta-dystroglycan. Identification of a central WW-like domain within caveolin family members, J. Biol. Chem. 275 (2000) 38048–38058.
[18] K.S. Song, P.E. Scherer, Z. Tang, T. Okamoto, S. Li, M. Chafel, C. Chu, D.S. Kohtz, M.P. Lisanti, Expression of caveolin-3 in skeletal, cardiac, and smooth muscle cells. Caveolin-3 is a component of the sarcolemma and co-fractionates with dystrophin and dystrophin-associated glycoproteins, J. Biol. Chem. 271 (1996) 15160–15165.
[19] P.E. Scherer, M.P. Lisanti, Association of phosphofructokinase-M with caveolin-3 in differentiated skeletal myotubes. Dynamic regulation by extracellular glucose and intracellular metabolites, J. Biol. Chem. 272 (1997) 20698–20705.
[20] C. Matsuda, Y.K. Hayashi, M. Ogawa, M. Aoki, K. Murayama, I. Nishino, I. Nonaka, K. Arahata, R.H. Brown Jr., The sarcolemmal proteins dysferlin and caveolin-3 interact in skeletal muscle, Hum. Mol. Genet. 10 (2001) 1761–1766.
[21] P.E. Scherer, R.Y. Lewis, D. Volonte, J.A. Engelman, F. Galbiati, J. Couet, D.S. Kohtz, E. van Donselaar, P. Peters, M.P. Lisanti, Cell-type and tissue-specific expression of caveolin-2. Caveolins 1 and 2 co-localize and form a stable hetero-oligomeric complex in vivo, J. Biol. Chem. 272 (1997) 29337–29346.
[22] F. Galbiati, D. Volonte, J.A. Engelman, P.E. Scherer, M.P. Lisanti, Targeted down-regulation of caveolin-3 is sufficient to inhibit myotube formation in differentiating C2C12 myoblasts. Transient activation of p38 mitogen-activated protein kinase is required for induction of caveolin-3 expression and subsequent myotube formation, J. Biol. Chem. 274 (1999) 30315–30321.
[23] C. Minetti, F. Sotgia, C. Bruno, P. Scartezzini, P. Broda, M. Bado, E. Masetti, P. Mazzocco, A. Egeo, M.A. Donati, D. Volonté, F. Galbiati, G. Cordone, F.D. Bricarelli, M.P. Lisanti, F. Zara, Mutations in the caveolin-3 gene cause autosomal dominant limb-girdle muscular dystrophy, Nat. Genet. 18 (1998) 365–368.
[24] F. Galbiati, D. Volonte, C. Minetti, D.B. Bregman, M.P. Lisanti, Limb-girdle muscular dystrophy (LGMD-1C) mutants of caveolin-3 undergo ubiquitination and proteasomal degradation, J. Biol. Chem. 275 (2000) 37702–37711.
[25] F. Galbiati, D. Volonte, J.B. Chu, M. Li, S.W. Fine, M. Fu, J. Bermudez, M. Pedemonte, K.M. Weidenheim, R.G. Pestell, C. Minetti, M.P. Lisanti, Transgenic overexpression of caveolin-3 in skeletal muscle fibers induces a Duchenne-like muscular dystrophy phenotype, Proc. Natl. Acad. Sci. USA 97 (2000) 9689–9694.
[26] A. Musarò, K. McCullagh, A. Paul, L. Houghton, G. Dobrowolny, M. Molinaro, E.R. Barton, H.L. Sweeney, N. Rosenthal, Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle, Nat. Genet. 27 (2001) 195–200.
[27] B.H. Jiang, M. Aoki, J.Z. Zheng, J. Li, P.K. Vogt, Myogenic signaling of phosphatidylinositol 3-kinase requires the serine-threonine kinase Akt/protein kinase B, Proc. Natl. Acad. Sci. USA 96 (1999) 2077–2081.
[28] G. Pallafacchina, E. Calabria, A.L. Serrano, J.M. Kalhovde, S. Schiaffino, A protein kinase B-dependent and rapamycin-sensitive pathway controls skeletal muscle growth but not fiber type specification, Proc. Natl. Acad. Sci. USA 99 (2002) 9213–9218.
[29] M.E. Coleman, F. DeMayo, K.C. Yin, H.M. Lee, R. Geske, C. Montgomery, R.J. Schwartz, Myogenic vector expression of insulin-like growth factor I stimulates muscle cell differentiation and myofiber hypertrophy in transgenic mice, J. Biol. Chem. 270 (1995) 12109–12116.
[30] K.M. Lai, M. Gonzalez, W.T. Poueymirou, W.O. Kline, E. Na, E. Zlotchenko, T.N. Stitt, A.N. Economides, G.D. Yancopoulos, D.J. Glass, Conditional activation of akt in adult skeletal muscle induces rapid hypertrophy, Mol. Cell. Biol. 24 (2004) 9295–9304.
[31] B.M. Scicchitano, L. Spath, A. Musaro, M. Molinaro, N. Rosenthal, C. Nervi, S. Adamo, Vasopressin-dependent myogenic cell differentiation is mediated by both Ca^{2+}/calmodulin dependent kinase and calcineurin pathways, Mol. Biol. Cell 16 (2005) 3632–3641.
[32] C. Nervi, L. Benedetti, A. Minasi, M. Molinaro, S. Adamo, Arginine-vasopressin induces differentiation of skeletal myogenic cells and up-regulation of myogenin and Myf-5, Cell. Growth Differ. 6 (1995) 81–89.
[33] S. Minotti, B.M. Scicchitano, C. Nervi, S. Scarpa, M. Lucarelli, M. Molinaro, S. Adamo, Vasopressin and insulin-like growth factors synergistically induce myogenesis in serum-free medium, Cell. Growth Differ. 9 (1998) 155–163.
[34] B.M. Scicchitano, L. Spath, A. Musaro, M . Molinaro, S. Adamo, C. Nervi, AVP induces myogenesis through the transcriptional activation of the myocyte enhancer factor 2, Mol. Endocrinol. 16 (2002) 1407–1416.
[35] A. Musaro, N. Rosenthal, Maturation of the myogenic program is induced by postmitotic expression of insulin-like growth factor I, Mol. Cell. Biol. 19 (1999) 3115–3124.
[36] A. Musaro, K.J. McCullagh, F.J. Naya, E.N. Olson, N. Rosenthal, IGF-1 induces skeletal myocyte hypertrophy through calcineurin in association with GATA-2 and NF-ATc1, Nature 400 (1999) 581–585.
[37] U. Grieshammer, D. Sassoon, N. Rosenthal, A transgene target for positional regulators marks early rostrocaudal specification of myogenic lineages, Cell 69 (1992) 79–93.
[38] W.E. Mitch, A.L. Goldberg, Mechanisms of muscle wasting. The role of the ubiquitin–proteasome pathway, N. Engl. J. Med. 335 (1996) 1897–1905, Review.
[39] S.H. Lecker, V. Solomon, W.E. Mitch, A.L. Goldberg, Muscle protein breakdown and the critical role of the ubiquitin–proteasome pathway in normal and disease states, J. Nutr. 129 (1999) 227S–237S, Review.