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*

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Caveolin-3 is the principal structural protein of caveolae membrane domains in striated muscle cells. Caveolin-3 mRNA and protein expression are dramatically induced during the differentiation of C2C12 skeletal myoblasts, coincident with myoblast fusion. In these myotubes, caveolin-3 localizes to the sarcolemma (muscle cell plasma membrane), where it associates with the dystrophin-glycoprotein complex. However, it remains unknown what role caveolin-3 plays in myoblast differentiation and myotube formation. Here, we employ an antisense approach to derive stable C2C12 myoblasts that fail to express the caveolin-3 protein. We show that C2C12 cells harboring caveolin-3 antisense undergo differentiation and express normal amounts of four muscle-specific marker proteins. However, C2C12 cells harboring caveolin-3 antisense fail to undergo myoblast fusion and, therefore, do not form myotubes. Interestingly, treatment with specific p38 mitogen-activated protein kinase inhibitors blocks both myotube formation and caveolin-3 expression, but does not affect the expression of other muscle-specific proteins. In addition, we find that three human rhabdomyosarcoma cell lines do not express caveolin-3 and fail to undergo myoblast fusion. Taken together, these results support the idea that caveolin-3 expression is required for myoblast fusion and myotube formation, and suggest that p38 is an upstream regulator of caveolin-3 expression.

Caveolae are 50–100-nm vesicular invaginations of the plasma membrane (1). It has been proposed that caveolae participate in vesicular trafficking events and signal transduction processes (1–5). Caveolin, a 21–24-kDa integral membrane protein, is a principal component of caveolae membranes in vivo (6–10). Caveolin is only the first member of a new gene family; as a consequence, caveolin has been re-termined caveolin-1 (11).

The mammalian caveolin gene family now consists of caveolins-1, -2, and -3 (4, 5, 11–13). Caveolins-1 and 2 are co-expressed and form a hetero-oligomeric complex (14) in many cell types, with particularly high levels in adipocytes, whereas expression of caveolin-3 is muscle-specific and found in both cardiac and skeletal muscle (15). Caveolin-3 is localized to the muscle cell plasma membrane (sarcolemma) where it forms a complex with dystrophin and its associated glycoproteins (15). However, under certain conditions caveolin-3 can be physically separated from the dystrophin complex (16). This indicates that, although caveolin-3 is dystrophin-associated, it is not absolutely required for the biogenesis of the dystrophin complex (16).

Caveolin-3 is most closely related to caveolin-1, based on protein sequence homology; caveolin-1 and caveolin-3 are ~65% identical and ~85% similar (see Tang et al. (13) for an alignment). However, caveolin-3 mRNA is expressed predominantly in muscle tissue types (skeletal muscle, diaphragm, and heart) (13). Identification of a muscle-specific member of the caveolin gene family has implications for understanding the role of caveolins in different muscle cell types (smooth, cardiac, and skeletal), as previous morphological studies have demonstrated that caveolae are abundant in these cells. A number of studies have highlighted the importance of caveolae and caveolins in the pathogenesis of Duchenne’s muscular dystrophy. More specifically, dystrophin has been localized to plasma membrane caveolae in smooth muscle cells using immunoelectron microscopy techniques (17), and skeletal muscle caveolae undergo characteristic changes in their size and distribution in patients with Duchenne’s muscular dystrophy, but not in other forms of neuromuscular dystrophies examined (18). This indicates that muscle cell caveolae may play an important role in muscle membrane biology.

In collaboration with Minetti and colleagues, we have recently identified an autosomal dominant form of limb-girdle muscular dystrophy (LGMD-1C) in two Italian families that is due to a deficiency in caveolin-3 expression. Analysis of their genomic DNA reveals two distinct mutations in the caveolin-3 gene: (i) a 9-base pair microdeletion that removes the sequence TFT from the caveolin-scaffolding domain, and (ii) a mis-sense mutation that changes a proline to a leucine (Pro → Leu) in the transmembrane domain (19). Both mutations lead to a loss of ~90–95% of caveolin-3 protein expression.

These results indicate that dramatic reductions in caveolin-3 can produce a disease phenotype in humans. However, it remains unknown whether caveolin-3 expression is required to generate or maintain the differentiated state of muscle cells. To
address this issue, we used an antisense approach to essentially ablate caveolin-3 expression in C2C12 cells, a well established murine skeletal myoblast cell line. Our results indicate that drastic down-regulation of caveolin-3 (to undetectable levels) prevents or inhibits myotube formation, but does not affect the expression of a panel of muscle-specific marker proteins. Thus, a deficiency in caveolin-3 expression seen in LGMD-1C patients could potentially slow the process of myotube formation in vivo and partially explain the pathogenic phenotype of this human genetic disorder.

**Experimental Procedures**

**Materials**—Antibodies and their sources were as follows: anti-caveolin-3 IgG (mAb 26; Ref. 15, gift of Dr. Roberto Campos-Gonzalez, Transduction Laboratories), anti-caveolin-2 IgG (mAb 65; Ref. 14, gift of Dr. Roberto Campos-Gonzalez), anti-caveolin-1 IgG (mAb 2297; Ref. 20, gift of Dr. Roberto Campos-Gonzalez), anti-caveolin-1 IgG (rabbit anti-peptide pAb N-20; directed against residues 2–21, Santa Cruz Biotechnology, Inc.), anti-troponin T (mAb, clone JLT-12, Sigma), anti-MHC (mAb, clone NO7.5.4D, Sigma), anti-dystrophin (N terminus, mAb, clone NCL-DYS3, Novo Castra), anti-b-dystroglycan (mAb, clone NCL-b-DG, Novo Castra), anti-p38 MAP kinase (pAb, New England Biolabs), and anti-phosphospecific p38 MAP kinase (pAb, New England Biolabs).

**Cell Culture**—C2C12–3 cells (21) were derived from a single colony of C2C12 cells (22) and display a more stable phenotype than the original C2C12 cell line. C2C12–3 myoblasts were cultured as described previously (21). Briefly, proliferating C2C12–3 cells were cultured in high mitogen medium (DMEM containing 15% fetal bovine serum and 1% chicken embryo extract) and induced to differentiate at confluence in low mitogen medium (DMEM containing 3% horse serum) (13, 15, 23). 

Overt differentiation was indicated by the assembly of multinucleated syncytia, which commenced 36–48 h after the cells were switched to low mitogen media. RD (ATCC/CCL-136), A673 (ATCC/CRL-1588), and HS729 (ATCC/HTB-153) cells were differentiated for 2 days in DMEM supplemented with 2% horse serum, glutamine, and antibiotics (penicillin and streptomycin).

**Construction of the Caveolin-3 Antisense Vector**—The full-length untagged cDNA encoding rat caveolin-3 (13) was inserted in the antisense orientation into an expression vector that was driven by the b-actin promoter (pCAGGS, gift of Dr. Armin Rehn, Ploegh Laboratory, Harvard Medical School, MA). The pCAGGS construct was co-transfected with a plasmid containing hygromycin resistance (pCB7).

**Establishment of Stable C2C12 Cell Lines Harboring Caveolin-3 Antisense**—C2C12 cells were transfected with caveolin-3 antisense vector using a modified calcium phosphate precipitation protocol. Resistant clones were selected using hygromycin B (200 lg/ml). Individual clones were isolated using cloning rings. Lysates from differentiated C2C12 were processed and assayed for reductions in the expression of caveolin-3 by immunoblotting. C2C12 cells were also transfected with empty vector alone as a control.

**p38 Inhibitor Treatment**—C2C12 cells were treated for the indicated period of time with 10 m amounts of SB203580, SB202190, or SB202474 (an inactive control compound) (Calbiochem, Inc.). Similar results were obtained at a concentration of 5 m.

**Phase Microscopy**—C2C12, RD, A673, and HS729 cells were grown in plastic tissue culture dishes and photographed using an inverted Nikon microscope.

**Immunoblotting Analysis**—Cellular proteins were resolved by SDS-PAGE (12.5% or 8% acrylamide) and transferred to nitrocellulose membranes. Blots were incubated for 2 h in TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.2% Tween 20) containing 2% powdered skim milk and 1% bovine serum albumin. After three washes with TBST, membranes were incubated for 2 h with the primary antibody (~1,000-fold diluted in TBST) and for 1 h with horseradish peroxidase-conjugated goat anti-rabbit/mouse IgG (~5,000-fold diluted). Proteins were detected using an ECL detection kit (Amersham Pharmacia Biotech).

**RESULTS**

**Targeted Down-regulation of Caveolin-3 Protein Expression in C2C12 Cells That Harbor Caveolin-3 Antisense**—In order to selectively down-regulate the expression of the caveolin-3 protein, we engineered an expression vector containing the untagged full-length caveolin-3 cDNA in the antisense orientation. For this purpose, we used the well established murine C2C12 skeletal myoblast cell line. Cultured C2C12 cells offer a convenient system to study skeletal myoblast differentiation. These cells can be induced to differentiate from myoblasts into myotubes bearing an embryonic phenotype in low mitogen medium over a period of 2 days. Briefly, proliferating C2C12 cells are cultured in high mitogen medium (DMEM containing 15% fetal bovine serum and 1% chicken embryo extract) and induced to differentiate at confluence in low mitogen medium (DMEM containing 3% horse serum). Overt differentiation is indicated by the assembly of multinucleated syncytia, which commences 36–48 h after the cells are switched to low mitogen media. In addition, we have previously shown that both mRNA and protein levels of caveolin-3 are dramatically induced during the course of differentiation of C2C12 cells from myoblasts to myotubes (13, 15).

These caveolin-3 antisense constructs were first tested in transient transfection assays with C2C12 cells and were found to significantly reduce the expression levels of endogenous caveolin-3 during myoblast differentiation, as compared with mock-transfected or vector alone controls (data not shown). Greater than the preliminary success of this approach in transient transfections, we decided to derive stable cell lines that harbor this caveolin-3 antisense construct.

Three C2C12 cell lines harboring caveolin-3 antisense were derived, and they all behaved similarly. As a consequence, one clone was selected for in depth analysis.

Fig. 1 shows a Western blot analysis of the expression of caveolin-3 in C2C12 cells harboring caveolin-3 antisense and untransfected control cells. Note that caveolin-3 levels are ef-
flectively reduced during the differentiation process. In addition, caveolin-1 and caveolin-2 levels were not affected by the expression of caveolin-3 antisense, demonstrating that the expression of caveolin-3 antisense selectively down-regulates the expression of caveolin-3. Importantly, C2C12 cells harboring vector alone did not show any changes in the levels of caveolin-3 expression (see below).

C2C12 Cells Harboring Caveolin-3 Antisense Express Normal Levels of Muscle-specific Marker Proteins—To investigate whether targeted down-regulation of the caveolin-3 protein overtly affects the differentiation process, we next evaluated the expression of a panel of muscle-specific marker proteins in C2C12 cells harboring caveolin-3 antisense. These markers included both cytoskeletal elements (troponin T and myosin heavy chain) and muscle-specific plasma membrane components (β-dystroglycan and dystrophin). Fig. 2 shows the results of this analysis. Interestingly, C2C12 cells harboring caveolin-3 antisense expressed normal levels of troponin T, myosin heavy chain, β-dystroglycan, and dystrophin, as compared with untransfected control C2C12 cells. These results clearly indicate that the process of differentiation is not overtly affected by targeted down-regulation of caveolin-3 expression.

C2C12 Cells Harboring Caveolin-3 Antisense Fail to Undergo Myoblast Fusion and Myotube Formation—Myoblast fusion and myotube formation is indicated by the assembly of multinucleated syncytia, which commences ~36–48 h after the C2C12 cells are switched to low mitogen media and can be observed morphologically.

Interestingly, C2C12 cells harboring caveolin-3 antisense failed to undergo myoblast fusion (Fig. 3A), despite the fact that they undergo differentiation normally and continue to express muscle-specific marker proteins that are a hallmark of normal adult muscle (Fig. 2). In striking contrast, C2C12 cells...
pathway is a prerequisite for the differentiation of 3T3-L1 fibroblasts to adipocytes (24), we next assessed the activation state of the p38 pathway during differentiation in C2C12 cells. For this purpose, we used immunoblotting with phosphospecific antibody probes that are routinely used to assess p38 activation. We observed that p38 MAP kinase activation occurred early during the differentiation program and was transient, with peak activity on day 2 (Fig. 5). In contrast, the protein levels of total p38 MAP kinase remain relatively constant, as seen using a phospho-independent antibody probe. Thus, these results are in agreement with our previous results with the adipocyte system (24).

Inhibition of p38 MAP Kinase Blocks Caveolin-3 Expression and Myotube Formation, but Does Not Affect the Expression of a Variety of Muscle-specific Marker Proteins—We next used a well established and highly selective p38 MAP kinase inhibitor (SB203580) to assess the role of p38 activation in the differentiation of C2C12 cells. Importantly, this inhibitor does not affect the activation of the p42/44 and the stress-activated protein/c-Jun N-terminal kinase MAP kinase pathways (see Ref. 24, and references therein). Using this approach with the adipocyte system, we recently showed that activation of p38 is required for achieving the differentiatied adipogenic phenotype and for up-regulation of the caveolin-1 protein product (24). Fig. 6 shows that treatment with the p38 inhibitor (SB203580) selectively blocks the expression of the caveolin-3 protein, but has little or no effect on the expression of other specific markers of the muscle cell plasma membrane or cytoskeletal elements. In addition, treatment with the p38 inhibitor SB203580 blocked myoblast fusion/myotube formation (Fig. 7A). However, addition of the p38 inhibitor SB203580 after 2 days of differentiation did not reverse myoblast fusion. Fig. 8 shows a direct comparison of the effects of caveolin-3 antisense or the p38 inhibitor SB203580 on myotube formation. Note that both treatments effectively block this process.

As additional controls for the effects of the p38 inhibitor SB203580, we also evaluated the effects of a second well characterized p38 inhibitor, SB202190, and a known related inactive control compound, SB202474. Our results indicate that the second p38 inhibitor (SB202190) effectively blocks caveolin-3 expression and myoblast fusion, while SB202474 is inactive as predicted (Figs. 6B and 7B). Virtually identical results were obtained after either 2 days or 5 days of differentiation.

Down-regulation of Caveolin-3 in Human Rhabdomyosarcoma Cell Lines—RD cells are a well established human rhabdomyosarcoma cell line. As caveolin-1 levels are down-regulated in a variety of transformed fibroblastic and human breast

Fig. 5. p38 MAP kinase activity is transiently up-regulated during C2C12 cell differentiation. Lysates were prepared from parental C2C12 cells after 0, 2, 3, or 4 days of differentiation. After SDS-PAGE and transfer to nitrocellulose, immunoblotting was performed with phospho-specific and phospho-independent antibody probes: (i) anti-activated p38 (upper panel) and (ii) anti-p38 (lower panel). Note the dramatic increase in activated p38 after 2 days of differentiation. Each lane contains equal amounts of total protein.

Fig. 4. Characterization of the phenotype of C2C12 cells harboring vector alone. A, Western blot analysis. Expression of troponin T (upper panel) and caveolin-3 (lower panel) in two independent clones harboring vector alone and in normal parental C2C12 cells is shown. Note that C2C12 cells harboring vector alone did not show reductions in the expression level of caveolin-3 or troponin T and behaved as parental C2C12 cells. Each lane contains equal amounts of total protein. B, morphological characterization. Left panels, cells grown in proliferation medium (Prolif); right panels, cells grown in differentiation medium (Diff). The results obtained with two representative clones are shown here. Note that C2C12 cells harboring vector alone show normal myotube formation after 2 days of differentiation, as compared with parental C2C12 cells.

harboring vector alone continue to express muscle-specific protein markers, caveolin-3, and undergo myotube formation normally (Fig. 4). These results indicate that targeted down-regulation of the caveolin-3 protein is sufficient to block myoblast fusion and subsequent myotube formation.

One possibility is that caveolin-3 down-regulation may cause a delay, rather than a block in myotube formation. To address this issue, we examined myoblast fusion after 5 days of differentiation. Even under these conditions, no myoblast fusion/myotube formation was observed with C2C12 cells that harbor caveolin-3 antisense (Fig. 3B), as compared with control C2C12 cells. These results are more consistent with the idea that down-regulation of caveolin-3 results in a block in myoblast fusion, rather than a delay.

Transient Activation of the p38 MAP Kinase Pathway Occurs during the Differentiation of C2C12 Myoblasts to Myotubes—As we recently observed that activation of the p38 MAP kinase

FIG. 4. Characterization of the phenotype of C2C12 cells harboring vector alone. A, Western blot analysis. Expression of troponin T (upper panel) and caveolin-3 (lower panel) in two independent clones harboring vector alone and in normal parental C2C12 cells is shown. Note that C2C12 cells harboring vector alone did not show reductions in the expression level of caveolin-3 or troponin T and behaved as parental C2C12 cells. Each lane contains equal amounts of total protein. B, morphological characterization. Left panels, cells grown in proliferation medium (Prolif); right panels, cells grown in differentiation medium (Diff). The results obtained with two representative clones are shown here. Note that C2C12 cells harboring vector alone show normal myotube formation after 2 days of differentiation, as compared with parental C2C12 cells.

harboring vector alone continue to express muscle-specific pro-
cancer cell lines (25–29), we next examined the expression of the caveolin-3 protein in RD cells. Interestingly, RD cells express muscle-specific markers (such as troponin T), but do not express the caveolin-3 protein product (Fig. 9A). In addition, they fail to undergo myoblast fusion (Fig. 9B). Thus, it appears that caveolin-3 expression is down-regulated during skeletal muscle cell transformation.

As a consequence of these observations with RD cells, we analyzed two additional ATCC cell lines derived from human rhabdomyosarcomas (A673 and Hs729) and the results are shown in Fig. 9 (A and B). Note that A673 and Hs729 cells do not express the caveolin-3 protein product and they fail to undergo myoblast fusion. Thus, our results indicate that in all three rhabdomyosarcoma-derived cell lines (RD, A673, and Hs729 cells), caveolin-3 levels are down-regulated and these cell lines fail to undergo myoblast fusion.
However, it is important to note that recombinant expression of caveolin-3 in RD cells was not sufficient to drive myoblast fusion and myotube formation (data not shown). These results indicate that although caveolin-3 expression may be required for or greatly facilitates myoblast fusion, caveolin-3 expression is clearly not sufficient to drive myoblast fusion in the context of RD cells.

**DISCUSSION**

LGMD-1C is an autosomal dominant form of limb-girdle muscular dystrophy that is genetically caused by mutations within the coding region of the caveolin-3 gene. In collaboration with Minetti and colleagues (19), we have recently identified two different families in Italy with this form of muscular dystrophy. In these patients, the levels of the caveolin-3 protein are reduced by $90$–$95\%$ as revealed by immunofluorescence and Western blot analysis. These results indicate that dramatic reductions in caveolin-3 can produce a disease phenotype in humans. However, it remains unknown whether caveolin-3 expression is required to generate or maintain the differentiated phenotype of muscle cells.

Here, we have directly addressed this issue by using an antisense approach to ablate caveolin-3 expression in C2C12 cells. We show that C2C12 cells harboring caveolin-3 antisense undergo differentiation and express normal amounts of four muscle-specific marker proteins. However, C2C12 cells harboring caveolin-3 antisense fail to undergo myoblast fusion and do not form myotubes. Thus, a deficiency in caveolin-3 expression may potentially slow the process of myotube formation in vivo, contributing to the pathogenesis of LGMD-1C.

Using phosphospecific antibody probes, we noted that p38 MAP kinase activation was transiently induced during the early phase of myoblast differentiation. Interestingly, treatment with a specific p38 inhibitor (either SB203580 or SB202190) blocked both myotube formation and caveolin-3 expression, but did not affect the expression of other muscle-specific proteins. These results support the idea that caveolin-3 expression is required for myoblast fusion and myotube formation, and suggest that p38 is an upstream regulator of caveolin-3 expression. These data also suggest that p38 MAP kinase activation and subsequent caveolin-3 expression at the muscle...
cell plasma membrane must play a critical role in myoblast fusion.

Recently, we and other laboratories have shown that activation of the p38 MAP kinase pathway occurs during a variety of differentiation processes. Inhibition of p38 activation effectively blocks these differentiation processes. These processes include the nerve growth factor-induced differentiation of PC12 cells into neuron-like cells (30), the conversion of 3T3-L1 fibroblasts to adipocytes (24), and the erythropoietin-mediated induction of red blood cell formation (31). In the case of 3T3-L1 cells, we have also shown that treatment with p38 MAP kinase inhibitors blocks the induction of caveolin-1 protein expression (24). Normally, both the caveolin-1 mRNA and protein levels are induced ~10–25-fold during the process of adipogenesis (14, 32). Thus, by analogy with p38-mediated regulation of caveolin-1 expression during adipogenesis, it is not completely unexpected to observe that inhibition of the p38 MAP kinase pathway prevents expression of the caveolin-3 protein and inhibits myotube formation.

Addendum—While our paper was being revised, two other groups reported the effects of p38 inhibition on C2C12 (33) and L5 (34) myoblast differentiation. As we observe here, they also found that p38 activation was required for myotube formation. However, they did not implicate p38-mediated induction of caveolin-3 in the process of myotube formation. In addition, their results with p38 inhibitors blocks the induction of caveolin-1 protein expression and inhibits myotube formation.

REFERENCES

1. Smart, E. J., Graf, G. A., McNiven, M. A., Sessa, W. C., Engelman, J. A., Scherer, P. E., Okamoto, T., and Lisanti, M. P. (1999) Mol. Cell Biol. 19, 7289–7304
2. Lisanti, M. P., Scherer, P., Tang, Z.-L., and Sargiacomo, M. (1994) Trends Cell Biol. 4, 231–233
3. Couet, J., Li, S., Okamoto, T., Scherer, P. S., and Lisanti, M. P. (1997) Trends Cardiovasc. Med. 7, 103–110
4. Okamoto, T., Schlegel, A., Scherer, P. E., and Lisanti, M. P. (1998) J. Biol. Chem. 273, 5419–5422
5. Engelman, J. A., Zhang, X. L., Galbiati, F., Volonte, D., Setgia, F., Pestell, R. G., Minetti, C., Scherer, P. E., Okamoto, T., and Lisanti, M. P. (1998) Am. J. Hum. Genet. 63, 1578–1587
6. Glenney, J. R., Jr. (1989) J. Biol. Chem. 264, 20163–20166
7. Glenney, J. R., and Soppet, D. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10517–10521
8. Glenney, J. R. (1992) FERS Lett. 314, 45–48
9. Rothberg, R. G., Heuser, J. E., Donzell, W. C., Ying, Y., Glenney, J. R., and Anderson, R. G. W. (1992) Cell 68, 673–682
10. Kurzchaia, T., Dupree, P., Parton, R. G., Kellner, R., Virta, H., Lehner, M., and Simons, K. (1992) J. Cell Biol. 118, 1003–1014
11. Scherer, P. E., Okamoto, T., Chun, M., Nishimoto, I., Lodish, H. F., and Lisanti, M. P. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 131–135
12. Parton, R. G. (1998) Curr. Opin. Cell Biol. 8, 542–548
13. Tang, Z., Scherer, P. E., Okamoto, T., Song, K., Chu, C., Kohzt, D. S., Nishimoto, I., Lodish, H. F., and Lisanti, M. P. (1998) J. Biol. Chem. 271, 2255–2261
14. Scherer, P. E., Lewis, R. Y., Volonte, D., Engelmann, J. A., Galbiati, F., Couet, J., Kohzt, D. S., van Donselaar, E., Peters, P., and Lisanti, M. P. (1997) J. Biol. Chem. 272, 29337–29346
15. Song, K. S., Scherer, P. E., Tang, Z., Okamoto, T., Li, S., Chafele, M., Chu, C., Kohzt, D. S., and Lisanti, M. P. (1996) J. Biol. Chem. 271, 15160–15165
16. Crosseie, R. H., Yamada, H., Venzke, D. P., Lisanti, M. P., and Campbell, K. P. (1998) FEBS Lett. 427, 279–282
17. North, A. J., Galanziewicz, R., Byers, T. J., Glenney, J. R., and Small, J. V. (1993) J. Cell Biol. 120, 1159–1167
18. Bonilla, E., Fishbeek, K., and Schotland, D. (1981) Am. J. Pathol. 104, 167–173
19. Minetti, C., Setgia, F., Bruno, C., Scartezzini, P., Broda, P., Iado, M., Masetti, E., Mazzocco, P., Egeo, A., Donati, M. A., Volonte, D., Galbiati, F., Cordone, G., Bricarelli, F. D., Lisanti, M. P., and Zara, F. (1998) Nat. Genet. 18, 365–368
20. Scherer, P. E., Tang, Z.-L., Chun, M., Sargiacomo, M., Lodish, H. F., and Lisanti, M. P. (1995) J. Biol. Chem. 270, 16395–16401
21. Cole, F., Fasy, T. M., Rao, S. S., de Peralta, M. A., and Kohtz, D. S. (1993) J. Biol. Chem. 268, 1580–1585
22. Blau, H., Chiu, C.-P., and Webster, C. (1983) Cell 32, 1171–1180
23. Scherer, P. E., and Lisanti, M. P. (1997) J. Biol. Chem. 272, 20698–20705
24. Engelman, J. A., Lisanti, M. P., and Scherer, P. E. (1998) J. Biol. Chem. 273, 32111–32120
25. Koleske, A. J., Baltimore, D., and Lisanti, M. P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1381–1385
26. Sager, R., Sheng, S., Anisowicz, A., Sotiropoulou, G., Zou, Z., Stenman, G., Suisitseh, K., Chen, Z., Hendrix, M. J. C., Pemberton, P., Raffi, K., and Ryan, K. (1994) Cold Spring Harbor Sym. Quant. Biol. LIX, 537–546
27. Engelman, J. A., Wykoff, C. C., Yasuhara, S., Song, K. S., Okamoto, T., and Lisanti, M. P. (1997) J. Biol. Chem. 272, 16374–16381
28. Engelman, J. A., Lee, R. J., Karnezis, A., Bearss, D. J., Webster, M., Siegel, P., Muller, W. J., Windle, J. J., Pestell, R. G., and Lisanti, M. P. (1998) J. Biol. Chem. 273, 20448–20455
29. Lee, S. W., Reimer, C. L., Oh, P., Campbell, D. B., and Schnitzer, J. E. (1998) Oncogene 16, 1391–1397
30. Moroska, T., and Nishida, E. (1998) J. Biol. Chem. 273, 24285–24288
31. Nagata, Y., Takahashi, N., Davis, R. J., and Todokoro, K. (1998) Blood 92, 1859–1869
32. Scherer, P. E., Lisanti, M. P., Baldini, G., Sargiacomo, M., Corley-Mastick, C., and Lodish, H. F. (1994) J. Cell Biol. 127, 1233–1243
33. Cuenda, A., and Cohen, P. (1999) J. Biol. Chem. 274, 4341–4346
34. Zetser, A., Gredinger, E., and Bengal, E. (1999) J. Biol. Chem. 274, 5193–5200