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Calcium regulates caveolin-1 expression at the transcriptional level

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ABSTRACT

Caveolin-1, an indispensable component of caveolae serving as a transformation suppressor protein, is highly expressed in poorly metastatic mouse osteosarcoma FBJ-S1 cells while highly metastatic FBJ-LL cells express low levels of caveolin-1. Calcium concentration is higher in FBJ-S1 cells than in FBJ-LL cells; therefore, we investigated the possibility that calcium signaling positively regulates caveolin-1 in mouse FBJ-S1 cells. When cells were treated with the calcium channel blocker nifedipine, cyclosporin A (a calcineurin inhibitor), or INCA-6 (a nuclear factor of activated T-cells [NFAT] inhibitor), caveolin-1 expression at the mRNA and protein levels decreased. RNA silencing of voltage-dependent L-type calcium channel subunit alpha-1C resulted in suppression of caveolin-1 expression. This novel caveolin-1 regulation pathway was also identified in mouse NIH 3T3 cells and Lewis lung carcinoma cells. These results indicate that caveolin-1 is positively regulated at the transcriptional level through a novel calcium signaling pathway mediated by L-type calcium channel/Ca^{2+}/calcineurin/NFAT.

1. Introduction

The integral membrane protein caveolin-1 is approximately 21–24 kDa and is found primarily in 50- to 100-nm flask-shaped invaginations called plasma membrane caveolae, where it acts as a scaffold to organize multiple molecular complexes that regulate a variety of cellular events [1,2]. Through the caveolin scaffolding domain, caveolin-1 binds to proteins that contain the caveolin-1 binding domain, making caveolin-1 indispensable for regulating the activities of multiple signaling molecules at the inner plasma membrane [1]. Caveolin-1 acts as a tumor suppressor or promoter in many different tumors, possibly because of its different interacting partners during tumor progression [3]. It has been proposed that caveolin-1 plays a tissue- and stage-specific modulatory role in tumors in vivo [4].

To investigate the mechanism of tumor metastasis, we obtained a set of non-metastatic and metastatic cells from an FBJ-virus-induced mouse osteosarcoma [5,6]. The poorly metastatic FBJ-S1 cells feature the expression of ganglioside GD1a [7], caveolin-1, and stromal interaction molecule 1 (Stim1) [8] while highly metastatic FBJ-LL cells are distinct in expressing matrix metallopeptidase 9 (MMP9) [9], Tumor necrosis factor α (TNF) [10], inducible NO synthase (NOS2) [11], and hepatocyte growth factor (HGF) [12]. In the present study, we have determined the internal calcium concentration of these cells using flow cytometry with Fluo 4-AM and found that the calcium concentration in FBJ-S1 cells was much higher than in FBJ-LL cells. These results prompted us to investigate whether caveolin-1 is regulated through calcium signaling. Caveolin-1 gene expression is transcriptionally down-regulated by three independent pathways [13]: Ras-p42/44 MAPK, non-receptor tyrosine kinases, and protein kinase A. This report is the first to describe the involvement of calcium signaling in the regulation of caveolin-1.

2. Methods

2.1. Cell lines and culture

The poorly metastatic mouse osteosarcoma cell line, FBJ-S1, was produced from FBJ virus-induced osteosarcoma of the BALB/c mouse [5]. FBJ-S1 cells express a high level of GD1a [7] and caveolin-1 [8]. Murine Lewis lung carcinoma and NIH 3T3 cell lines were purchased from the Riken gene bank (Ibaraki, Japan). The FBJ cells and NIH 3T3 cells were maintained in RPMI-1640 medium

Abbreviations: Cacna1, L-type calcium channel alpha-1 subunit (c, etc.); HBSS, Hank's buffered salt solution; NFAT, nuclear factor of activated T-cells; PI, propidium iodide; qPCR, quantitative real-time PCR; RT-PCR, reverse transcriptase-polymerase chain reaction; Stim1, stromal interaction molecule 1.

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(GIBCO, Invitrogen Life Technologies Corporation, NY, USA) and the Lewis lung carcinoma cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen Life Technologies Corporation), each containing 10% newborn calf serum (GIBCO, Invitrogen Life Technologies Corporation, Auckland, New Zealand), 100 units/mL penicillin, and 100 μg/mL streptomycin. Cells were cultured as reported previously [8]. Cell proliferation was measured using the WST-1 assay [9,11] with a cell counting kit (Dojindo, Kumamoto, Japan) according to the manufacturer's protocol.

2.2. Chemicals

Nifedipine (L-type calcium channel blocker, dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate), calcium ionophore A23187, cyclosporin A, and mouse anti-actin monoclonal antibody were obtained from Sigma–Aldrich, USA. INCA-6 (nuclear factor of activated T-cells [NFAT] activation inhibitor) and rabbit anti-caveolin-1 polyclonal antibody were from Santa Cruz Biotechnology, Inc., (CA, USA). Horseradish peroxidase-linked anti-mouse IgG secondary antibody and anti-rabbit IgG secondary antibody were obtained from Cell Signaling (MA, USA).

2.3. Flow cytometric analyses

FBJ cells were assayed for calcium concentration using Fluo 4-AM dye (Dojindo) [14]. The cells were seeded with an initial inoculation of 1×10^6 cells in a 6-cm dish; 24 h later, adherent cells were detached with 0.02% trypsin in EDTA/PBS(−) and washed with PBS(−). Cells were incubated with 4 μM Fluo 4-AM at 37 °C for 30 min in Hank's buffered salt solution (HBSS) followed with washing by PBS (−). In some experiments, the cells were incubated with propidium iodide (PI, 5 μg/mL) with Fluo 4-AM. Cells were suspended in PBS(−) buffer and analyzed by flow cytometry with a BD FACScan Calibur.

2.4. RNA extraction and RT-PCR

Cells were harvested and total RNA was extracted from 1×10^6 cells using the QIAGEN RNeasy Kit (Hilden, Germany) according to the manufacturer's instructions. The mRNA levels for the genes of interest were in most cases determined semi-quantitatively using reverse transcriptase-polymerase chain reaction (RT-PCR) following synthesis of first-strand cDNA from total RNA using a Takara RNA PCR Kit (AMV) Ver.3.0 (TaKaRa Bio, Dalian, China) with oligo dT-adaptor primers and 1 μg total RNA as the template, as previously described [8]. RT-PCR primers were designed using Primer 3 (v. 0.4.0) software [Web-1] and synthesized by Invitrogen Life Technologies Corporation (Beijing, China). The primer sequences were as follows:for Rpl13 (Rpl), sense 5′-CATCAGGCCCATCGTGA GGT-3′ and antisense 5′-GCAGCTTCCTTCGCCCTTTT-3′; for caveolin-1, sense 5′-CTACAAGCCCAACAACAAGGC-3′ and antisense 5′-AGGAAGCTCTTGATGCACGGT-3′; for the calcium channel voltage-dependent L-type alpha 1S subunit (Cacna1s), sense 5′-TCCCG GAGGCTAGCTCCTTC-3′ and antisense 5′-AGCACGATGTTCCCGA TGGT-3′; for the calcium channel voltage-dependent L-type alpha 1C subunit (Cacna1c), sense 5′-CCATGCTGACGGTGTTCCAG-3′ and antisense 5′-GCAAGCCTGGCTCCACAGTT-3′; for the calcium channel voltage-dependent L-type alpha 1D subunit (Cacna1d), sense 5′-CATGGTCCCCCTCCTTCACA-3′ and antisense 5′-GCTGCTGTTTT TCCCGAAGC-3′; and for the calcium channel voltage-dependent L-type alpha 1F subunit (Cacna1f), sense 5′-CCCCTTCCCTGAGGA CGACT-3′ and antisense 5′-TCGAGTCCGATGATGGCGTA-3′. Following 2% agarose electrophoresis and ethidium bromide staining for 1 h, the intensity of the stained band was assessed with a Bioprofile Bio 1D image analyzer (Vilber Lourmat, Marne-la-Vallee, France) at 312 nm. DNA content was divided by that of Rpl and usually expressed as 1 for control experiments [8].

2.5. Real time PCR

Total RNA was incubated with the PrimeScript® RT reagent kit contents with gDNA Eraser (TaKaRa Bio) followed by a reverse-transcription reaction. The resulting cDNA was used for amplification by real-time quantitative polymerase chain reaction (qPCR) in a 96-well format with SYBR® Premix Ex Taq™ (TaKaRa Bio) and a Stratagene MX3000P system (Angient, China). The data obtained were incorporated into LinRegPCR software [Web-2], and template amounts were calculated. The template amount of the gene of interest was divided by that of the standard gene (Rpl13) and usually expressed as 1 for control experiments. The primers used for qPCR were chosen by Primer5 [Web-3], as follows: for caveolin-1, sense 5′-GTCAACCGCGACCCCAAGC-3′ and antisense 5′-GTAAAT GCCCCAGATGAGTGCC-3′; for Rpl 13, sense 5′-AAAGTGGCTCGCACC ATCGG-3′ and antisense 5′-CCATTCGGAGACTGGCAAAAGC-3′.

2.6. SDS–PAGE and Western blot

A total of 1×10^6 cells were lysed in 1 mL lysis buffer (50 mM HEPES, NaCl 137 mM, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM $Na_4P_2O_7$, 10 mM NaF, 2 mM EDTA, 10% glycerol, 2 mM vanadate, 10 mg/mL aprotinin, 2 mM PMSF, 25 mM glycerophosphate, 1% Triton X-100) at 4 °C for 10 min. Protein concentration was determined using the BioRad Protein Assay kit. After being boiled in the double-strength sample buffer at 100 °C for 5 min, the lysate was loaded onto a 12% SDS–polyacrylamide gel. Following electrophoresis, the gel was blotted and subjected to Western blotting as detailed previously [8].

2.7. Double-stranded siRNA and transient transfection

Transient transfection was performed with the transfection reagent Lipofectamine 2000 (Invitrogen Corporation), according to the manufacturer's instructions. FBJ-S1 cells (0.6×10^6) were seeded in 60-mm dishes, and after overnight incubation, double-stranded siRNA (0.2 nmol) was introduced into the cells for 48 h. Then, RNA or protein extraction was performed [12]. The sequences of the double-stranded siRNA (GenePharma Corporation, Shanghai, China) used in this study were chosen using an Invitrogen siRNA Wizard v3.1 [Web-4], as follows: Cacna1c seq 1, 5′-GAA GCAGCAACUAGAAGAATT-3′; Cacna1c seq 2, 5′-GAUCACCCAGG CAGAAGACTT-3′; and negative control, 5′-UUCUCCGAACGUGUC ACGUTT-3′.

2.8. Statistical analysis

All values are given as the mean ± SD. Statistical analyses were performed with the Student's *t*-test using Microsoft Excel. The level of significance is indicated in figures when more than three independent experiments were performed.

3. Results

3.1. Calcium concentration in FBJ-S1 cells is much higher than in FBJ-LL cells

Poorly metastatic FBJ-S1 cells express a higher level of caveolin-1 than highly metastatic FBJ-LL cells [8]. FBJ cells were subjected to flow cytometry using Fluo 4-AM to stain calcium inside cells. Poorly metastatic FBJ-S1 cells (Fig. 1A, upper panel) showed much higher staining with Fluo 4-AM than highly metastatic FBJ-LL cells

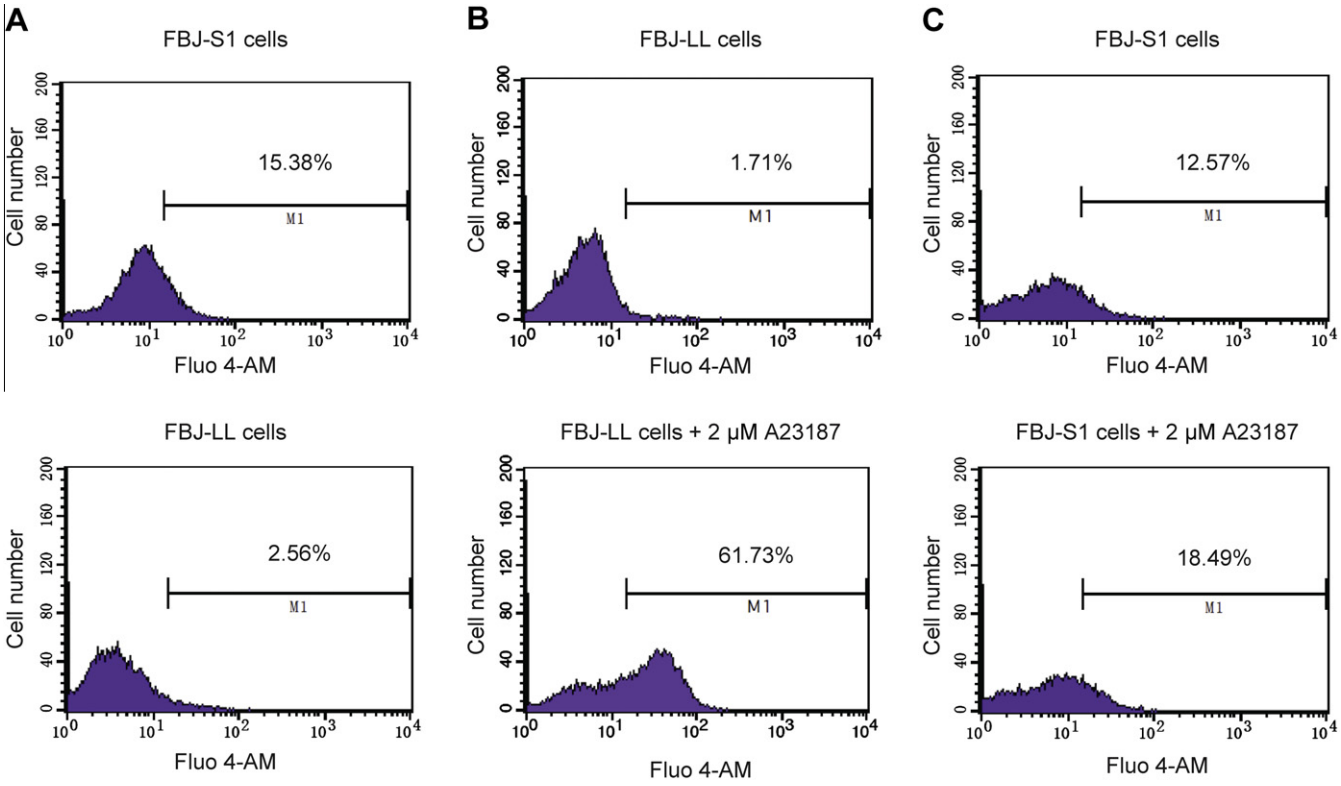

Fig. 1. Calcium concentration is much higher in FBJ-S1 cells than in FBJ-LL cells. FBJ-S1 (A, upper panel) and FBJ-LL (A, lower panel) were stained with Fluo 4-AM and analyzed by flow cytometry. The cells incubated without Fluo 4-AM did not show positive signal (not shown). In (B) and (C), immediately after the addition of 2 μM of the calcium ionophore A21387 to FBJ-LL (B) or FBJ-S1 (C) cell suspension, the cells were subjected to flow cytometry. Figures are the representative of more than three independent experiments.

(Fig. 1A, lower panel). PI staining of two cell lines showed no difference in the intensity of the PI-stained cells (Supplementary Fig. 1A and B), indicating that intense staining of FBJ-S1 cells with Fluo 4-AM was not because of an increase in dead cells. To show that Fluo 4-AM intensity reflected the inner calcium concentration, FBJ-S1 (Fig. 1B) and FBJ-LL (Fig. 1C) cells were treated with 2 μM of the calcium ionophore A23187. Fluorescence intensity was instantaneously increased significantly in FBJ-LL cells (Fig. 1B) compared to FBJ-S1 (Fig. 1C) cells; thus, the above results clearly indicate that FBJ-S1 cells had a much higher calcium concentration than FBJ-LL cell line, raising the possibility that caveolin-1 is regulated through calcium concentration.

3.2. Caveolin-1 is regulated by L-type voltage-dependent Ca^{2+} channels

Calcium concentration inside the animal cells, roughly 10^{-7} M, is strictly regulated. Calcium influx is mediated through several channels; voltage-dependent Ca ion channels [15], SOCE (store-operated Ca ion entry) channels [16], IP3-gated Ca ion-release channels [17], and the ryanodine receptor [18]. Among these channels, L-type voltage-dependent Ca ion channels are implicated in the majority of the calcium ion influx. If caveolin-1 expression is regulated by calcium, suppression of voltage-dependent Ca^{2+} channel activity would be predicted to affect caveolin-1 expression. FBJ-S1 cells were incubated with nifedipine, an L-type Ca^{2+} channel blocker [19], at 50 μM, for 12 or 24 h. The effects of nifedipine on FBJ-S1 cell growth are shown in Fig. 2A. Fifty micromole nifedipine suppressed caveolin-1 as well as Cacna1c (an isoform expressed in FBJ-S1 cells) expression at the mRNA (Fig. 2B) and protein (Fig. 2C) levels, suggesting that Ca^{2+} regulation through Ca^{2+} channels is essential for caveolin-1 expression. Fig. 2D shows that the calcium concentration of FBJ-S1 cells decreased on treatment of the cells with nifedipine even within 30 min following the treatment.

Several L-type voltage-dependent Ca^{2+} channels have been reported in mammals, namely, Cacna1s, Cacna1c, Cacna1d, and Cacna1f. Expression of these channel proteins at the mRNA level was determined by RT-PCR. As evident from RT-PCR amplification in the FBJ-S1 cell line (Supplementary Fig. 2), only the gene encoding isoform c of the L-type Ca^{2+} channel was detectably expressed in FBJ-S1 cells; thus, this gene was the target of further studies. Double-stranded siRNA directed against mouse Cacna1c sequences 1 and 2 inhibited expression of Cacna1c (Fig. 2E), and expression of caveolin-1 was decreased at the mRNA (Fig. 2E) and protein levels (Fig. 2F), supporting the notion that Cacna1c positively regulates caveolin-1 expression at the transcriptional level.

3.3. Calcium signal regulates caveolin-1 expression

To provide further evidence that calcium signaling is indispensable to caveolin-1 expression, FBJ-S1 cells were incubated with the calcineurin inhibitor cyclosporin A at 10 μM, which inhibited cell growth by 25% in 24 h (Fig. 3A). Cyclosporin A treatment of FBJ-S1 cells decreased caveolin-1 mRNA expression to 31%, as assessed by RT-PCR (Fig. 3C), and caveolin-1 protein to 30% (Fig. 3D), suggesting involvement of the calcineurin/a nuclear factor of activated T-cells (NFAT) pathway in caveolin-1 regulation. Calcineurin activated by calmodulin may dephosphorylate NFAT, which is translocated to the nucleus to activate the related gene [20]. FBJ-S1 cells thus were next treated with the NFAT activation inhibitor INCA-6. The viability of FBJ-S1 cells with INCA-6 was 117 and 82% at 10 and 20 μM, respectively, after 24 h (Fig. 3B). Treatment of the cells at 10 and 20 μM INCA-6 resulted in suppression of caveolin-1 mRNA to 60 and 50%, respectively, at the mRNA level (Fig. 3E)

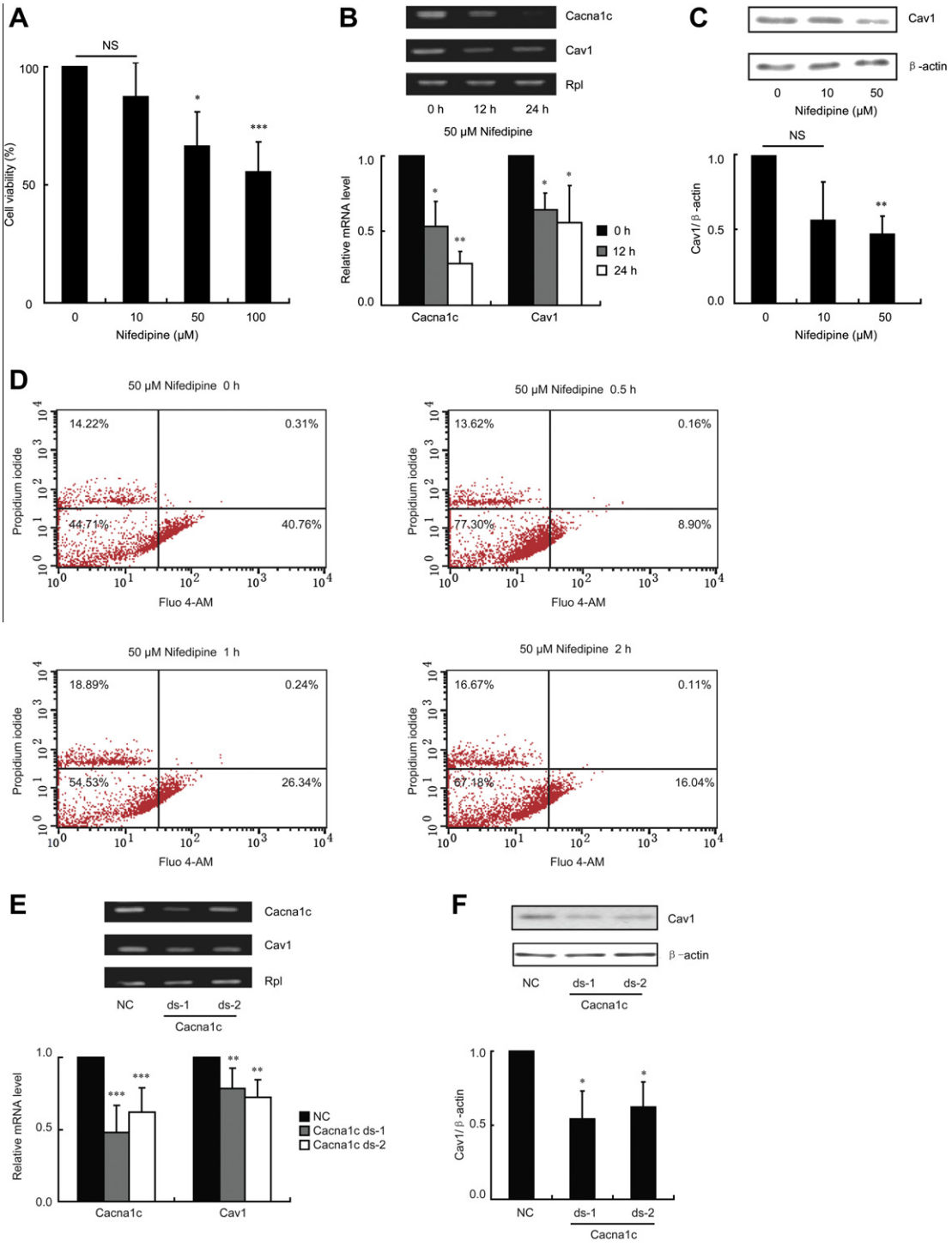

Fig. 2. Caveolin-1 is regulated via Cacna1c in FBJ-S1 cells. FBJ-S1 cells were treated with nifedipine (a calcium channel inhibitor) (A) at the concentration indicated for 24 h in a 96-well plate and cell number was assessed by WST-1 assay (A). FBJ-S1 cells were treated with 50 μM nifedipine for 12 or 24 h, and Cacna1c and caveolin-1 (Cav1) expression was determined by RT-PCR with Rpl13 (Rpl) as the standard (B). FBJ cells were treated with 10 or 50 μM nifedipine for 24 h and caveolin-1 expression was determined by Western blot (C). In (D), FBJ cells were treated with 50 μM nifedipine for the indicated time, and calcium concentration inside the cells was measured using flow cytometry with Fluo 4-AM. FBJ-S1 cells were transfected with double-stranded siRNAs directed toward Cacna1c (two sequences, ds-1 and ds-2) or a scrambled control RNA (NC); expression of Cacna1c and caveolin-1 mRNA (E) were determined by RT-PCR and caveolin-1 protein expression, by Western blot with β-actin as the control (F). In (B), (C), (E), and (F), the representative results are shown in the upper panel; data in the lower panel are the means of at least three independent experiments. NS, not significant; $^{*}P < 0.05$, and $^{**}P < 0.01$, and $^{***}P < 0.001$, relative to the control.

and 35 and 23% at the protein level (Fig. 3F), respectively. This result supports the idea that the calcineurin/NFAT pathway is involved in caveolin-1 regulation and the novel notion that calcium positively regulates caveolin-1 expression. Considering all of the results together, caveolin-1 expression appears to be regulated through the Cacna1c/Ca^{2+}/calcineurin/NFAT signaling pathway.

3.4. Caveolin-1 is regulated through the L-type calcium channel/calcineurin/NFAT signaling pathway in mouse NIH 3T3 and Lewis lung carcinoma cell lines

To determine whether the newly found pathway regulating caveolin-1 is universal, mouse NIH 3T3 (Fig. 4A and B for mRNA

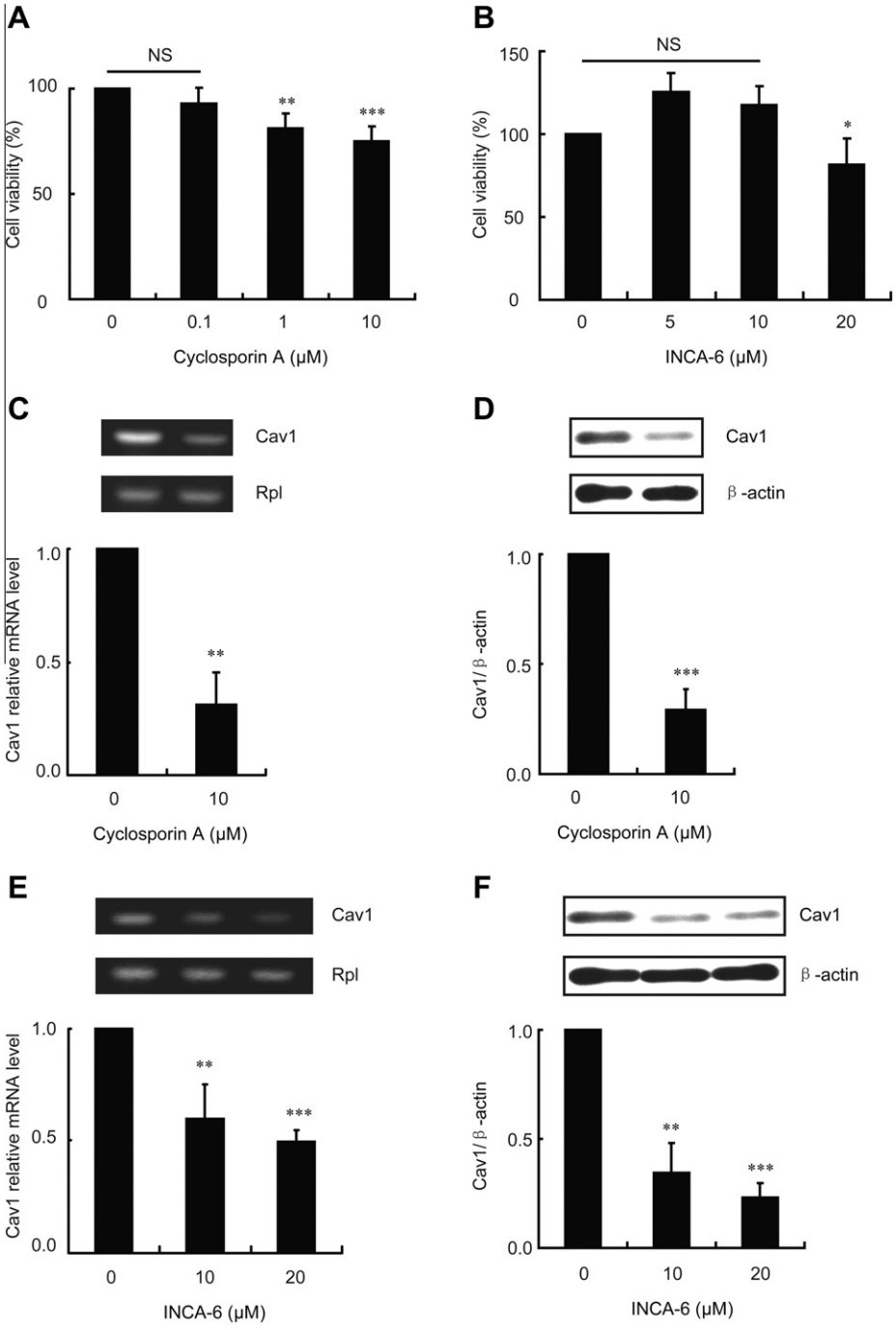

Fig. 3. Caveolin-1 is regulated by a signal mediated through Cacna1c/Ca^{2+}/calcineurin/NFAT. FBJ-S1 cells were incubated with different concentrations of cyclosporin A (A) or INCA-6 for 24 h (B) and the number of cells assessed by WST-1. FBJ-S1 cells were incubated with 10 μM cyclosporin A for 12 h or 24 h to determine caveolin-1 mRNA expression (C) or protein (D) by RT-PCR or Western blot, respectively. FBJ-S1 cells also were treated with 10 and 20 μM INCA-6 for 12 h (E) and 24 h (F), and caveolin-1 (Cav1) expression at mRNA (E) and protein (F) levels was estimated. The representative results from (C) to (F) are shown in the upper panel, and data in the lower panel are the means of three independent experiments: NS, not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, relative to the control.

protein respectively) and Lewis lung carcinoma cells (Fig. 4C–E) were treated with the calcium channel inhibitor nifedipine, calcineurin inhibitor cyclosporin A, or NFAT inhibitor INCA-6. The effect on cell growth of each chemical at the concentrations used in this study is given in Supplementary Fig. 3A for NIH 3T3 and in Fig. 4 for Lewis cells. Caveolin-1 mRNA expression of NIH 3T3 cells was measured by qPCR with results comparable to those obtained by semi-quantitative PCR (Supplementary Fig. 4D). As clearly shown in Fig. 4, these treatments suppressed caveolin-1 expression at the mRNA and protein levels as they did in FBJ-S1 cells. All of these data indicate that caveolin-1 is regulated through the calcium channel in NIH 3T3 and Lewis cells and thus that this newly found caveolin-1-regulating pathway is not specific to FBJ-S1 cells.

4. Discussion

The results of this work point to calcium channel regulation of caveolin-1, a protein that plays a key role in cancer progression and metastasis. Originally thought to be a tumor suppressor

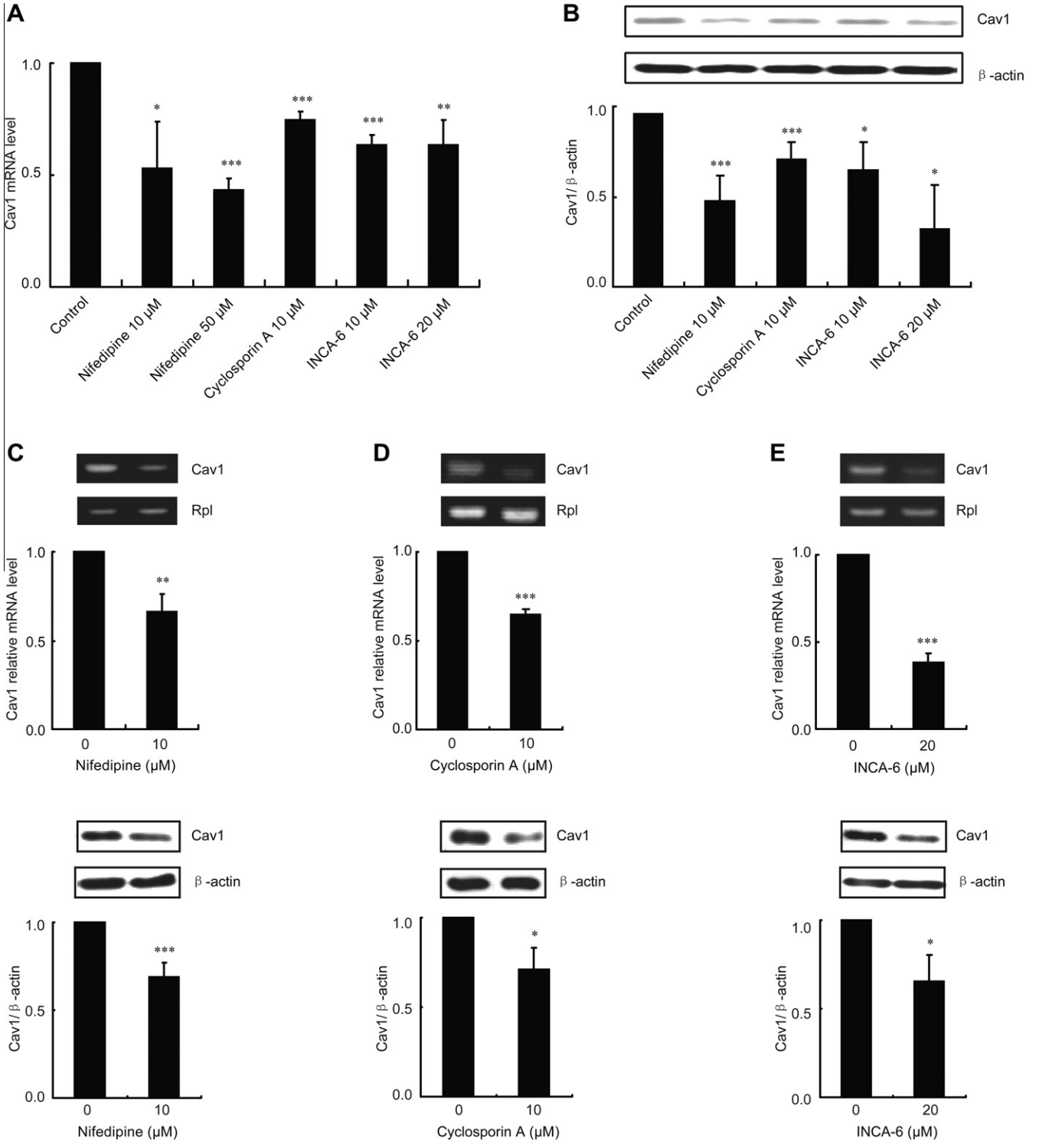

Fig. 4. Caveolin-1 is regulated in the same way in NIH 3T3 and Lewis cells as in FBJ-S1 cells. NIH 3T3 cells were incubated with 10 and 50 μM nifedipine, 10 μM cyclosporin A, or 10 and 20 μM INCA-6 for 12 h and assayed for caveolin-1 (Cav1) mRNA expression by qPCR (A). NIH3T3 cells were incubated with 10 μM nifedipine, 10 μM cyclosporin A, or 10 and 20 μM INCA-6 for 24 h and assayed for caveolin-1 protein expression by Western blot (B). Lewis cells were incubated with 10 μM nifedipine (C), 10 μM cyclosporin A (D), and 20 μM INCA-6 (E) for 12 h for mRNA (upper panels) or 24 h for protein (lower panel) measurements, by RT-PCR and by Western blot, respectively. The representative results from (B) to (E) are shown in the upper panel, and data in the lower panel are the means of three independent experiments: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, relative to the control.

protein, caveolin-1 was later found to promote tumorigenesis in the prostate [21], and it was positively associated with tumor dedifferentiation in a subset of high-grade bladder cancer [22]. Engagement of caveolin-1 as a promoter or suppressor of tumor metastasis is strongly determined by the specific cellular context and by caveolin-1-regulating signaling at the molecular level; thus, its functions and regulation have been extensively studied. Lisanti et al. revealed that the mitogen-activated protein kinase (MAPK) and protein kinase A pathways regulate caveolin-1 expression [13]. Among murine FBJ cells, highly metastatic FBJ-LL cells express

less caveolin-1 compared to poorly metastatic FBJ-S1 cells [8], suggesting its role in suppressing metastasis. The present study is the first to describe the involvement of calcium signaling in caveolin-1 regulation in mouse cell lines.

Ca^{2+} functions as an important signaling messenger in many organisms, from the moment of fertilization to the final moment of life. Ca^{2+}-mediated signaling pathways also play important roles in carcinogenesis, as in the transformation of normal cells to cancerous cells, tumor formation and growth, invasion, angiogenesis, and metastasis [23]. Calcium channels additionally mediate the influx of Ca^{2+} into the cell upon membrane polarization. The gene Cacna1c encodes an alpha-1 subunit of a voltage-dependent calcium channel, which consists of 24 transmembrane segments and forms the pore through which Ca^{2+} passes into the cell. L-type Ca^{2+} channels are constituted by a central pore-forming $\alpha 1$ subunit associated with different accessory subunits ($\alpha 2$-δ, β, and γ). The L-type Ca^{2+} channel regulation is tissue specific; over 10 different types of L-type Ca^{2+} channels have been cloned and characterized, and their expression differs among tissues [24]. To date, four different L-type $\alpha 1$ subunits have been cloned; among four genes that encode $\alpha 1$ subunits of L-type Ca^{2+} channels, only Cacna1c (Cav-1.2) has been identified in FBJ-S1 cells (Supplementary Fig. 2). We expect to investigate further the mechanism by which Cacna1c is activated in poorly metastatic FBJ-S1 cells compared to highly metastatic FBJ-LL cells.

Some other possible explanations for these findings of calcium regulation of caveolin-1 require discussion. Nifedipine, known as a calcium channel blocker, suppressed caveolin-1 expression in FBJ-S1 cells at 10 and 50 μM, but it could be argued that suppression of molecules other than the calcium channel brought about this decrease in caveolin-1, since 10 and 50 μM of nifedipine decreased cell viability to 86 and 66% (Fig. 2A), respectively, at 24 h. However, successful suppression of caveolin-1 by RNA silencing of Cacna1c clearly indicates a function of calcium channels and accordingly that calcium signaling is indispensable to caveolin-1 expression. Furthermore, the increase in calcium ion concentrations inside the cell might activate calmodulin, resulting in activation of calcineurin, whose activity is blocked by cyclosporin A. FBJ-S1 cells were treated with 10 μM cyclosporin A, which decreased cell viability to 75% in 24 h (Fig. 3A). Suppression of caveolin-1 expression by cyclosporin A suggests that calcium signaling may regulate caveolin-1 expression.

Calcineurin also interacts, however, with myeloid differentiation primary response gene 88, toll-like receptor adaptor molecule 1, toll-like receptor 2, and toll-like receptor 4 [25], and cyclosporin A works not only on calcineurin but also on FOS-like antigen 2 [26], mitogen-activated protein kinase kinase kinase 12 [27], and PI3 K signaling [28]. Therefore, involvement of NFAT in caveolin-1 regulation was investigated using its inhibitor, INCA-6. With this treatment, caveolin-1 mRNA expression was suppressed to 60% at 10 μM and 50% at 20 μM without affecting cell viability significantly.

Thus, the Cacna1c/Ca^{2+}/calcineurin/NFAT signaling pathway appears to regulate caveolin-1 expression, and this study confirmed the involvement of the L-type Ca^{2+} channel in positive regulation of caveolin-1. Expression of both caveolin-1 and Stim1 is enhanced in poorly metastatic ganglioside GD1a-enriched FBJ-S1 cells while highly metastatic GD1a-deficient FBJ-LL cells express lower amounts of both molecules [8]. Stim1, a calcium sensor located at the endoplasmic reticulum membrane and reportedly a metastasis suppressor gene [29], may also be regulated via calcium. This idea awaits further study, as does the possibility that caveolin-1 has an NFAT binding sequence or a binding site for transcription factors that is activated by NFAT.

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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.bbrc.2012.08.079.

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