Recombinant Expression of Caveolin-1 in Oncogenically Transformed Cells Abrogates Anchorage-independent Growth*

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Introduction of caveolin-1 expression in v-Abl-transformed and H-Ras (G12V)-transformed NIH 3T3 cells abrogated the anchorage-independent growth of these cells in soft agar and resulted in the de novo formation of caveolae as seen by transmission electron microscopy. Consistent with its antagonism of Ras-mediated cell transformation, caveolin-1 expression dramatically inhibited both Ras/MAPK-mediated and basal transcriptional activation of a mitogen-sensitive promoter. Using an established system to detect apoptotic cell death, it appears that the effects of caveolin-1 may, in part, be attributed to its ability to initiate apoptosis in rapidly dividing cells. In addition, we find that caveolin-1 expression levels are reversibly down-regulated by two distinct oncogenic stimuli. Taken together, our results indicate that down-regulation of caveolin-1 expression and caveolae organelles may be critical to maintaining the transformed phenotype in certain cell populations.

Caveolae are plasma membrane-attached vesicular organelles. Caveolin-1, a 21–24-kDa integral membrane protein, is a principal component of caveolae membranes in vivo. Both caveolae and caveolin are most abundantly expressed in terminally differentiated cells: adipocytes, endothelial cells, and muscle cells. Conversely, caveolin-1 mRNA and protein expression are lost or reduced during cell transformation by activated oncogenes such as v-abl and H-ras (G12V); caveolae are absent from these cell lines. However, its remains unknown whether down-regulation of caveolin-1 protein and caveola organelles contributes to their transformed phenotype.

Here, we have expressed caveolin-1 in oncogenically transformed cells under the control of an inducible-expression system. Regulated induction of caveolin-1 expression was monitored by Western blot analysis and immunofluorescence microscopy. Our results indicate that caveolin-1 protein is expressed well using this system and correctly localizes to the plasma membrane. Induction of caveolin-1 expression in v-Abl-transformed and H-Ras (G12V)-transformed NIH 3T3 cells abrogated the anchorage-independent growth of these cells in soft agar and resulted in the de novo formation of caveolae as seen by transmission electron microscopy. Consistent with its antagonism of Ras-mediated cell transformation, caveolin-1 expression dramatically inhibited both Ras/MAPK-mediated and basal transcriptional activation of a mitogen-sensitive promoter. Using an established system to detect apoptotic cell death, it appears that the effects of caveolin-1 may, in part, be attributed to its ability to initiate apoptosis in rapidly dividing cells. In addition, we find that caveolin-1 expression levels are reversibly down-regulated by two distinct oncogenic stimuli. Taken together, our results indicate that down-regulation of caveolin-1 expression and caveolae organelles may be critical to maintaining the transformed phenotype in certain cell populations.

Caveolae are cell surface specializations that represent a subcompartment of the plasma membrane (1, 2). Morphologically, they appear as 50–100 nm vesicular structures near or attached to the plasma membrane (3, 4). Although caveolae are present in most cell types, they are most abundant in terminally differentiated cells such as adipocytes, endothelial cells, type I pneumocytes, and skeletal muscle cells (reviewed in Ref. 5). During adipocyte differentiation, the number of caveolae increases ~10-fold until they represent up to 20% of the surface area of the plasma membrane (6); similarly, both caveolin mRNA and protein are induced ~20–25-fold during this process (7, 8). The exact function of caveolin remains unknown; however, they are thought to participate in cellular transport processes and signal transduction (9–11).

Caveolin, a 21–24-kDa integral membrane protein, is a principal component of caveolae membranes (12–16). Recently, a family of caveolin-related proteins has been identified; caveolin-1 has been re-termed caveolin-1 (8, 17–20). It has been proposed that caveolin family members function as scaffolding proteins (21) to organize and concentrate specific lipids (cholesterol and glycosphingolipids (22–24)) and lipid-modified signaling molecules (Src-like kinases, H-Ras, eNOS, and G-proteins (22, 25–29)) within caveolae membranes. These and other signaling molecules appear to be tightly associated as a discrete complex with caveolin-1 as shown using a polyhistidine-tagged form of caveolin for detergent-free affinity purification of caveolae membranes (26).

Based on these and other observations, we and others have proposed the “caveolae signaling hypothesis,” which states that caveolar localization of certain inactive signaling molecules could provide a compartmental basis for their regulated activation and explain cross-talk between different signaling pathways (10, 30–32). In support of this idea, caveolin-1 binding can functionally suppress the GTPase activity of heterotrimeric G-proteins and inhibit the kinase activity of Src-family tyrosine kinases through a common caveolin domain, termed the caveolin-scaffolding domain (25–27). Thus, we have suggested that caveolin may function as a negative regulator of many different classes of signaling molecules through the recognition of specific caveolin-binding motifs (33).

Modification and/or inactivation of caveolin-1 expression appears to be a common feature of the transformed phenotype. Historically, caveolin was first identified as a major v-Src substrate in Rous sarcoma virus-transformed cells (34). Based on this observation, Glennen and co-workers (34) have proposed that caveolin may represent a critical target during cell transformation. In direct support of this notion, caveolin-1 mRNA and protein expression are reduced or absent in NIH 3T3 cells transformed by a variety of activated oncogenes (v-abl, Bcr-abl,

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EXPERIMENTAL PROCEDURES

Materials—mAb 1 2297 directed against caveolin-1 was the generous gift of Dr. John R. Glennon (Transduction Laboratories). The mAb 9E10 was provided by the Harvard Monoclonal Antibody Facility (Cambridge, MA). The cdNA for caveolin-1 was as we described previously (30). The NIH 3T3 cell line expressing a temperature-sensitive form of v-Abl was the generous gift of Dr. Naomi Rosenberg (Tufts University School of Medicine). A variety of other reagents was purchased commercially: donor calf serum, fetal bovine serum, pre-stained protein markers (Life Technologies, Inc.); IPTG (dioxane-free; Sigma and Calbiochem, Inc.); hygromycin B (Calbiochem, Inc.); SenPlaque low-melting temperature-agarose (American Bioanalytical); and lissamine rhodamine-conjugated goat anti-mouse IgG (Zymed, Inc.); anti-myec (polyclonal) IgG (MBL, Inc.). MEK (PD 98059) and p38 MAP kinase inhibitors (SB202190 and SB203580) were purchased from Calbiochem, Inc.

Cell Culture—Normal and transformed NIH 3T3 cells were cultured in Dulbecco’s modified Eagle’s medium containing glutamine, antibiotics, and 10% donor calf serum. 

Transfection and Selection of Stable Cell Lines—For inducible expression of caveolin-1, we used the LacSwitch Inducible Mammalian Expression system (Stratagene, Inc.). Briefly, a previously described C-terminally myc-tagged form of caveolin-1 (37) was subcloned into the NorI site of the vector pOP13CAT containing modified sequences from the lac operon, generating pOP13-Cav-1. v-Abl-transformed and H-Ras (G12V)-transformed NIH 3T3 cells were co-transfected with pOP13-Cav-1 and p35S (a vector encoding the lae-repressor and hygromycin resistance), as suggested by the manufacturer. After selection in medium supplemented with 150 μg/ml hygromycin, resistant colonies were picked by trypsinization using cloning rings. Individual clones were screened for IPTG-based induction of recombinant caveolin-1 expression by Western blot analysis using both anti-caveolin-1 (mAb 2297) and anti-myec (polyclonal) IgG. Induction was carried out for 20–40 h in medium containing 5 mM IPTG, as suggested by the manufacturer (Stratagene, Inc.).

Western Blot Analysis—Cells were extracted for 45 min on ice with Tris-buffered saline (10 mM Tris, pH 8.0, 0.15 mM NaCl) containing 60 mM octylglucoside (30, 38). The protein content of these extracts was determined using a BCA protein assay kit using bovine serum albumin as the standard. After SDS-polyacrylamide gel electrophoresis and transfer to nitrocellulose, blots were probed with anti-caveolin IgG (mAb 2297; 1/200) or anti-myec IgG (polyclonal; 1/500). Approximately 50 μg of cell extract was routinely loaded per lane. Bound IgG were visualized with horseradish peroxidase-conjugated secondary antibodies and the ECL system (Amersham and Pierce, Inc.). The relative amount of caveolin expressed in transformed cells was determined by comparison with standard dilutions of an extract of normal NIH 3T3 cells.

Immunoﬂuorescence—All reactions were performed at room temperature. v-Abl-transformed and H-Ras (G12V)-transformed NIH 3T3 cells harboring inducible caveolin-1 expression were plated onto coverslips and cultured in normal growth medium with or without 5 mM IPTG for 20–40 h. Cells were then briefly washed three times with PBS and fixed for 30 min in PBS containing 4% paraformaldehyde (8, 17, 18, 37, 39). Cells were then permeabilized by incubation with 0.1% Triton X-100 in PBS containing 0.5% bovine serum albumin for 10 min. After permeabilization, the cells were then successively incubated with PBS, 0.5% bovine serum albumin containing: (i) a 1:300 dilution of mAb 9E10 directed against the myc-epitope; and (ii) lissamine rhodamine B sulfonyl chloride-conjugated goat anti-mouse antibody (5 μg/ml). Incubations with primary and secondary antibodies were 60 min each. Cells were washed three times with PBS between incubations. Coverslips were mounted on slides with Slow-Fade anti-fade reagent and observed under a Bio-Rad MR600 confocal fluorescence microscope.

Transmission Electron Microscopy—Cells were fixed with glutaraldehyde, post-fixed with OsO4, and stained with uranyl acetate and lead citrate as described (30, 35). Samples were examined under a JEOL transmission electron microscope and photographed at a magnification of × 25,000 (30, 35). Caveolae were identified by their characteristic flask-shape, size (50–100 nm), and location at or near the plasma membrane (1–4).

Assay for Anchorage-independent Growth—Growth in soft agar was assayed as we described previously with minor modifications (35). Briefly, 2.5 × 103 cells of each cell line were suspended in 3 ml of Dulbecco’s modified Eagle’s medium containing glutamine, antibiotics, and 0.33% SeaPlaque low-melting temperature-agarose. These cells were plated over a 2 ml layer of solidified Dulbecco’s modified Eagle’s medium containing 10% donor calf serum and 0.5% agarose, and cells were allowed to settle to the interface between these layers at 37 °C. After 20 min, the plates were allowed to harden at room temperature for 30 min before returning to 37 °C. The plates were fed every 2–3 days by adding with 2 ml of medium containing 0.33% agarose. After 7–10 days, colonies were photographed under low magnification (× 4 or 6). In the case of IPTG induction, cells were induced with 5 mM IPTG for 20–40 h before plating; the agarose contained 22 mM IPTG to adjust for restricted diffusion in a semi-solid matrix.

c-fos Activation Assay—Activation of c-fos promoter activity was measured as we described previously (40). Briefly, CHO cells were co-transfected with a c-fos promoter-CAT plasmid and a plasmid encoding H-Ras (G12V). To evaluate the possible effect of caveolin on Ras activation, the caveolin-1 cdNA (pcB-7-Cav-1) or vector alone (pcB-7) was included during the co-transfection. CAT activity was measured essentially as described previously (40).

Apoptosis—The TUNEL assay was performed with a kit distributed by Oncor, as described previously (41, 42). Briefly, F11 cells or COS-7 cells (~4 × 105 cells/well) were seeded onto glass coverslips with 24-well dish. After transfection with the pcB-7 vector alone or with pcB-7-Cav-1, the cells on glass coverslips were processed according to the manufacturer’s instructions.

RESULTS

Caveolin-1 Expression Is Regulated by Oncogenic Stimulation—Caveolin-1 mRNA and protein levels are down-regulated in NIH 3T3 cells transformed by a variety of activated oncogenes, including v-abl/ and H-Ras (G12V) (35). However, it remains unclear whether reductions in caveolin-1 levels are a direct consequence of the oncogenic stimulus. Alternatively, cells that express reduced levels of caveolin-1 may simply be preferentially selected for oncogenic transformation.

To address this issue, we have employed an NIH 3T3 cell line expressing a temperature-sensitive form of v-Abl (43). This form of v-Abl contains two missense mutations engineered within its kinase domain that render the kinase inactive at the
non-permissive temperature (39 °C). As shown in Fig. 1, shift to the non-permissive temperature abrogates tyrosine phosphorylation of its cellular substrates; caveolin-1 levels are induced during inactivation of the kinase and return to levels seen in normal NIH 3T3 cells (Fig. 1, panel B). These results demonstrate that caveolin-1 levels can be down-regulated by an oncogenic stimulus. Normal and v-Abl transformed NIH 3T3 cells are shown for comparison; note that this temperature shift does not affect the activity of activated MEK or other serine/threonine protein kinases, including Raf-1, MAPK, JNK, cyclin A/cdk2, protein kinase A, and protein kinase C. Treatment with PHP does not affect the expression of caveolin-1 protein (not shown). As the identical construct clearly indicate that down-regulation of caveolin-1 expression in Ras-transformed cells is mediated by constitutive activation of the MEK/MAP kinase (p44 and p42) pathway. In contrast, treatment with specific inhibitors of p38 MAP kinases (SB202190 or SB203580 (45)) had no effect on caveolin-1 expression levels although these compounds markedly changed the morphology of the Ras-transformed cells.

Recombinant Expression of Caveolin-1 in Oncogenically Transformed NIH-3T3 Cells—As caveolin-1 expression is down-regulated during cell transformation, it has been previously suggested that caveolin-1 could function as a candidate transformation suppressor protein (35, 36). To explore this possibility, we proceeded to stably transfected v-Abl-transformed and H-Ras (G12V)-transformed NIH 3T3 cells with the caveolin-1 cDNA under the control of a constitutive cytomegalovirus-based promotor. However, none of the selected clones expressed the caveolin-1 protein (not shown). As the identical construct has been expressed well in other non-transformed mammalian cell lines such as Madin-Darby canine kidney cells (37), we speculated that expression of caveolin-1 in transformed cells could negatively affect their growth.

To test this hypothesis, we next transfected v-Abl-transformed and H-Ras (G12V)-transformed NIH 3T3 cells with the caveolin-1 cDNA under the control of an inducible mammalian expression system (LacSwitch; see “Experimental Procedures”). In this system, addition of 5 mM IPTG (isopropyl-β-D-thiogalactoside) to the culture media allows for regulated expression of the inserted gene product. IPTG is a non-hydrorlyzable galactose analog that is non-toxic to NIH 3T3 cells and other mammalian cells at concentrations up to 130 mM (46, 47). As shown in Fig. 2, this system was successfully employed to express caveolin-1 in transformed NIH-3T3 cells. Caveolin-1 expression was induced dramatically by incubation with 5 mM IPTG.
IPTG for 20–40 h; similar expression levels of caveolin-1 were obtained for both v-Abl-transformed and H-Ras (G12V)-transformed NIH 3T3 cells. The relative amount of caveolin-1 expressed in these transformed cells was estimated by comparison with standard dilutions of an extract of normal NIH 3T3 cells. Our results indicate that these IPTG-induced transformed cells express ~1/2 the level of caveolin-1 observed in normal NIH 3T3 cells. Thus, recombinant expression of caveolin-1 using this inducible system does not result in overexpression relative to normal endogenous levels.

Induction of caveolin-1 expression was also monitored by immunofluorescence microscopy. Fig. 3 shows that after IPTG induction of caveolin-1 expression, caveolin-1 appeared as discrete micropatches within the body of the cell and along the plasma membrane. As this staining pattern is virtually identical to the pattern observed in normal fibroblasts (8, 16, 37), these results provide an indication that caveolin-1 is correctly localized when expressed in oncogenically transformed cells. Induction of caveolin-1 protein expression also resulted in the de novo formation of caveolae in these transformed cell lines as seen by transmission electron microscopy (Fig. 4).

Induction of Caveolin-1 Expression Abrogates the Anchorage-independent Growth of Transformed Cells—Previous studies have indicated that caveolin-1 expression levels inversely correlate with the ability of transformed NIH 3T3 cells to grow in soft agar; for example, v-Abl transformed NIH 3T3 cells which express the least amount of caveolin-1 (less than 1% of normal NIH 3T3 cells) formed the largest colonies in soft agar (35). However, it is not known whether recombinant expression of caveolin-1 within these cells can suppress the transformed phenotype.

Thus, v-Abl-transformed and H-Ras (G12V)-transformed NIH 3T3 cells harboring inducible caveolin-1 were analyzed for their ability to propagate and form colonies in soft agar. Fig. 5A shows that in both cases caveolin-1 expression abrogates the growth of these transformed cells in soft agar. In the case of v-Abl, induction of caveolin-1 expression dramatically decreased the size of the colonies formed; in addition, colonies appeared to reach a certain maximal size (~15–20 cells) after which they appear to undergo “cell death” (Fig. 5A, upper panel; see also Fig. 5B for a higher magnification view).

In the case of H-Ras (G12V), induction of caveolin-1 expression dramatically decreased both colony size and the efficiency of colony formation; quantitation revealed that colony formation was inhibited ~3–4-fold (Fig. 5A, lower panel). However, caveolin-1 expression in H-Ras (G12V) transformed cells did not result in appreciable cell death of colonies. This may be due to the fact that H-Ras (G12V)-transformed cells are known to form colonies that are smaller than v-Abl transformed cells. It is important to note that IPTG did not affect the growth of parental v-Abl-transformed and H-Ras (G12V)-transformed NIH 3T3 cells evaluated in parallel.

Caveolin-1 Expression Inhibits Ras-mediated Transcriptional Activation of the c-fos Promoter—How does caveolin-1 expression abrogate the anchorage-independent growth of H-Ras (G12V)-transformed NIH 3T3? One possibility is that caveolin-1 expression inhibits certain downstream effectors of activated Ras preventing Ras-mediated transcriptional activation via the MAPK pathway.

To test this hypothesis, we next exploited an established assay to measure the effect of caveolin-1 expression on the transcriptional activation of the c-fos promoter by activated Ras (H-Ras (G12V) (40). Activation of the Ras/MAPK pathway (specifically Erk1 and Erk2) induces transcription from the
c-fos serum response element by phosphorylating transcription factors Elk-1 and SAP-1 which form a ternary complex (48).

In this assay system, CHO cells are co-transfected with a plasmid containing activated Ras (H-Ras (G12V)) and a plasmid harboring the c-fos protomer attached to a “reporter,” chloramphenicol acetyltransferase (CAT). CAT activity is then followed as a measure of Ras-mediated transcriptional activation. Fig. 6 shows that caveolin-1 expression (pCB-7-Cav-1) dramatically inhibited Ras-mediated transcriptional activation, as compared with the corresponding empty vector alone (pCB-7). In addition, caveolin-1 mediated-inhibition reached levels below the normal basal levels observed without activated Ras. These results are consistent with the idea that induction of caveolin-1 expression plays a negative regulatory role in the mitogenic response, as caveolin-1 expression inhibits Ras/MAPK-mediated transcriptional activation of a mitogen-sensitive promoter.

Caveolin-1 Expression and Apoptotic Cell Death—As induction of caveolin-1 expression in v-Abi transformed cells appeared to result in visible cell death of colonies grown in soft agar, we wondered whether caveolin-1 expression can also induce apoptosis. To evaluate this possibility, we employed an established assay system to detect apoptotic cell death (41, 42). The caveolin-1 cDNA (pCB-7-Cav-1) or vector alone (pCB-7) were transiently expressed in F11 cells. Nucleosomal DNA fragmentation in these cells was directly assessed by the TUNEL assay (terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate (dUTP) nick-end labeling) (41, 42).

Fig. 7 shows that transfection with the vector alone induced little or no DNA fragmentation after 36 h. In contrast, transfection with the caveolin-1 cDNA resulted in marked nucleosomal DNA fragmentation. As we previously described, transfection with another transmembrane protein (normal Alzheimer’s precursor protein) failed to induce nucleosomal fragmentation within the same assay system (41, 42). Thus, it appears that recombinant expression of caveolin-1 is sufficient to induce nucleosomal DNA fragmentation, a classic hallmark of apoptotic cell death.

**DISCUSSION**

v-Abi is the single protein encoded by the Abelson murine leukemia virus that is sufficient to mediate cell transformation (43, 49, 50). In contrast to its cellular homologue (c-Abi), v-Abi is a constitutively activated tyrosine kinase (43, 49). Cellular transformation by v-Abi is mediated, at least in part, by the Ras-MAP kinase pathway (51, 52). For example, reversion of the v-Abi-induced transformed phenotype can be achieved by: (i) expressing a dominant negative form of H-Ras (51) or (ii) micro-injecting anti-Ras IgG (52). Although the mechanism by which v-Abi activates Ras remains unknown, it has been suggested that v-Abi can interact directly with Shc thereby initiating the formation of a complex with Grb2-mSos and ensuing Ras-Raf activation (49). A mutationally activated form of Ras, most commonly G12V, is found in up to 30% of all human

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**FIG. 5. Induction of caveolin-1 expression abrogates the growth of transformed cells in soft agar.** A, v-Abi-transformed and H-Ras (G12V)-transformed NIH 3T3 cells expressing inducible caveolin-1 were analyzed for their ability to propagate and form colonies in soft agar. Upper panels, v-Abi transformed cells; lower panels, H-Ras (G12V)-transformed cells. Note that in both cases that induction of caveolin-1 expression (+ IPTG) abrogates their growth in soft agar. In the case of v-Abi-transformed cells, induction of caveolin-1 expression also resulted in visible cell death of the majority of colonies (seen as black dots) after reaching a certain maximal size. A higher magnification view is presented in B. IPTG had no effect on the growth of untransfected parental v-Abi-transformed and H-Ras (G12V)-transformed NIH 3T3 cells that lack inducible caveolin expression (not shown). Colonies were photographed under low magnification (v-Abi, × 4; H-Ras (G12V), × 6). B, v-Abi-transformed NIH 3T3 cells expressing inducible caveolin-1 undergo cell death after reaching a certain maximal colony size in soft agar. Left, v-Abi-transformed colony without caveolin-1 induction; right, v-Abi-transformed colonies after induction of caveolin-1 expression. Note that induction of caveolin-1 expression dramatically decreased the size of the colonies formed; in addition, colonies appeared to reach a certain maximal size (~15–20 cells) after which they appear to undergo “cell death” (see also A, right upper panel). Colonies were photographed under high magnification (× 30).
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Fig. 6. Caveolin-1 expression inhibits Ras-mediated transcriptional activation of a mitogen-sensitive promoter. CHO cells (35-mm dishes) were co-transfected with a c-fos promoter-CAT plasmid (0.5 μg) and a plasmid encoding activated Ras (H-Ras (G12V) in pECE; 0.5 μg) or the empty vector (pECE; 0.5 μg). To evaluate the possible effect of caveolin-1 expression on Ras-mediated transcriptional activation, the caveolin-1 cDNA (pCB-7-Cav-1; 0.5 μg) or vector alone (pCB-7; 0.5 μg) was included during co-transfection. CAT activity was measured at 48 h post-transfection; the values represent the mean ± S.D. of three independent experiments. The 100% CAT activity is the activity of the c-fos promoter-CAT transfected alone (9.9 ± 0.13 milliunits/mg protein). Note that caveolin-1 expression dramatically inhibited Ras-mediated transcriptional activation, as compared with the corresponding empty vector. Also, caveolin-1 mediated-inhibition reached levels below the normal basal levels observed without activated Ras.

Fig. 7. Caveolin-1 expression can induce apoptosis. TUNEL analysis of fragmented nucleosomal DNA in F11 cells 36 h post-transfection with the caveolin-1 cDNA (pCB-7-Cav-1, lower panel) or vector alone (pCB-7, upper panel). The 3'-OH ends of fragmented DNA are specifically stained brown by exogenously added terminal deoxynucleotidyltransferase and digoxigenin-labeled dUTP (see "Experimental Procedures"). Nucleosomally fragmented DNA was finally visualized using anti-digoxigenin IgGs conjugated to alkaline phosphatase. Virtually identical results were obtained by transfection of COS-7 cells (not shown).

Here, we have examined the potential role of caveolin-1 in v-Abl and H-Ras (G12V)-mediated cell transformation using NIH 3T3 cells as a model system. Caveolin-1 mRNA and protein expression are dramatically down-regulated in response to cellular transformation by these and other activated oncogenes (35). In the case of v-Abl, we show here that caveolin-1 protein expression is down-regulated as a consequence of the v-Abl-mediated oncogenic stimulus by employing a temperature sensitive form of v-Abl. For Ras-transformed cells, we demonstrate that caveolin-1 protein expression is down-regulated in response to constitutive activation of the MEK/MAPK pathway. In addition, we show that regulated induction of caveolin-1 expression in v-Abl and H-Ras (G12V)-transformed NIH 3T3 cells abrogates their growth in soft agar.

What is the mechanism behind caveolin's transformation suppressor activity? One possibility is that caveolin-1 expression antagonizes the effects of activated Ras by acting on some of its downstream elements. In support of this idea, we find using an independent approach in CHO cells that caveolin-1 expression can suppress Ras-mediated transcriptional activation using the c-fos promoter linked to CAT as a reporter. A second mechanism may involve the initiation of apoptosis. In the case of v-Abl-transformed cells, induction of caveolin-1 expression led to apparent cell death of colonies after reaching a certain maximal size of ~15–20 cells. However, this phenomenon was not observed during induction of caveolin-1 expression in H-Ras (G12V)-transformed cells. To independently assess whether caveolin-1 expression is capable of initiating apoptosis, we employed an established cell system to detect nucleosomal DNA fragmentation via the TUNEL assay. Our results indicate that caveolin-1 expression is sufficient to initiate nucleosomal DNA fragmentation, a hallmark of apoptotic cell death.

Several elements critical for mitogenic signaling have been previously localized to plasma membrane caveolae in normal cells, including epidermal growth factor and platelet-derived growth factor receptor tyrosine kinases (62–64), G-protein coupled receptors (39, 65, 66), G-proteins (26, 30, 31, 67, 68), protein kinase C (31, 62), Src-family tyrosine kinases (26, 30, 31, 69, 70), eNOS (28, 29), H-Ras (26, 63), Raf kinase (63), 14-3-3 proteins (64), MAP kinase (31, 64), Shc (64), Grb-2 (64), mSos-1 (64), and Nck (64). In addition, evidence has been presented suggesting that caveolin-1 interacts directly with many of these proteins: G-protein α subunits (8, 17, 22, 25, 33), Src-family tyrosine kinases (27), H-Ras (26), eNOS (71, 72), and Shc (73). Binding to caveolin-1 appears to be mediated by a cytoplasmic membrane-proximal region of caveolin-1, termed the caveolin scaffolding domain (27, 33). A peptide encoding this caveolin-1 scaffolding domain can suppress the GTPase activity of heterotrimeric G-proteins and abolish the tyrosine kinase activity of Src family kinases, indicating that this caveolin-1 domain can recognize and inactivate more than one class of signaling molecules (8, 17, 25, 27).

These findings with caveolin-1 are analogous to another fam-
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