Possible Role of Calponin h1 as a Tumor Suppressor in Human Uterine Leiomyosarcoma

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Background: Calponin h1, a basic actin-binding protein capable of inhibiting smooth muscle contraction, is a constitutive element of smooth muscle cells. However, in leiomyosarcoma (a type of smooth muscle neoplasm of the uterus), reduced expression of calponin h1 is observed, as we have reported previously. In this study, we sought to assess the effects (in vitro and in vivo) of increasing calponin h1 expression in leiomyosarcoma cells.

Methods: A plasmid containing a human calponin h1 complementary DNA and a bacterial neomycin-resistance gene was transfected into the human leiomyosarcoma cell lines SKN and SK-LMS-1 by electroporation. Southern blotting, reverse transcription–polymerase chain reaction analysis, western blotting, and immunohistochemistry were used to confirm DNA transfer and expression of the calponin h1 protein in neomycin-resistant clones. We characterized the morphology of calponin h1-transfected cells, and we evaluated their proliferative activity and tumorigenicity by use of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay, an anchorage-independent growth assay, and a nude mouse tumorigenicity assay.

Results: The morphology of calponin h1-transfected cells in culture resembled that of cultured normal myometrial smooth muscle cells. With SK-LMS-1 cells, proliferation of calponin h1-transfection cells was reduced to 69% of control; with SKN cells, calponin h1 transfection reduced proliferation to 70% of control. In assays of anchorage-independent growth and in vivo tumorigenicity, both growth and tumorigenicity were statistically significantly reduced in calponin h1-transfected leiomyosarcoma cells.

Conclusions: Calponin h1 may function as a tumor suppressor in leiomyosarcoma. Clinically, transfer of a calponin h1 complementary DNA into poorly differentiated leiomyosarcoma cells may be of potential therapeutic value through induction of a normal, differentiated cellular phenotype. [J Natl Cancer Inst 1999;91:790–6]

Leiomyosarcoma constitutes only about 1.3% of uterine cancers and one quarter of uterine sarcomas, but it is important because it is an extremely malignant neoplasm, exhibiting high rates of local recurrence and distant metastasis. In fact, the 5-year survival rate for patients with uterine leiomyosarcoma is 15%–25% (1). The introduction of a new therapy for leiomyosarcoma of the uterus is urgently needed because neither current radiotherapy nor chemotherapy is effective. However, the molecular and genetic changes underlying the neoplastic transformation of uterine smooth muscle cells (SMCs) have not been fully characterized. It has been reported that the expression of cytoskeletal molecules associated with SMC differentiation is reduced in malignant smooth muscle neoplasms (2–6). Those reports suggested that malignant smooth muscle neoplasms are composed of cells with less-differentiated smooth muscle phenotypes.

Basic calponin is a 34-kd member of the calponin family and was isolated originally as an F-actin-binding protein from chicken gizzard and bovine aortic SMCs (7,8). The gene encoding this protein, i.e., the calponin-h1 gene, expresses the originally isolated isoform of calponin, which is produced specifically in SMCs (9–11). Calponin h1 is a heat-stable, calmodulin- and actin-/tropomyosin-binding protein that is capable of inhibiting smooth muscle contraction (12–15). Interestingly, when expressed constitutively, it may also act to inhibit cell proliferation independently of its effect on contractility (16,17). In addition, expression of the calponin gene is regulated dur-
ing the development and differentiation of SMCs (18,19).

We have recently demonstrated that among the smooth muscle tumors of the uterus, only leiomyosarcomas exhibit a reduced expression of calponin h1 (2). This association between reduced expression of calponin h1 and malignant transformation of the uterine SMC suggested to us that calponin h1 may function as a tumor suppressor in leiomyosarcoma. In the present study, we tried to determine whether or not transfection of the human calponin h1 gene might affect the proliferative activity and tumorigenicity of leiomyosarcoma cells.

**Materials and Methods**

**Cell culture and DNA transfection.** The human leiomyosarcoma cell line, SKN (20), provided by I. Ishiwata, was cultured in Ham’s F12 medium with 15% fetal calf serum (FCS) (Biological Industries, Kibbutz Beth-Haemek, Israel) and 1% antibiotic–antifungal solution. Incubation was carried out at 37 °C in 5% CO2 in air in DMEM with 10% FCS and 1% antibiotic–antifungal solution. Incubation was carried out at 37 °C under 5% CO2 in air. Each of these cell lines is negative for the expression of calponin h1 by both immunohistochemistry and reverse transcription–polymerase chain reaction (RT–PCR) analysis (2). Wild-type human calponin h1 complementary DNA (cDNA) (22) was subcloned into a pCMV-neo-Bam vector. In brief, a human calponin h1 expression plasmid, including a cDNA insert (1560 base pairs [bp]) that contains the entire coding sequence for human calponin h1, was cloned into the BamHI site of the vector pCMV-neo-Bam, a mammalian expression vector carrying the neomycin acetyltransferase gene (neo), which confers neomycin resistance. The human calponin h1 cDNA was under the control of a cytomegalovirus (CMV) promoter. Twenty micrograms either of the human calponin h1 cDNA construct or of the pCMV-neo-Bam vector was transfected into each of the leiomyosarcoma cell lines SKN (4 × 106 cells) and SK-LMS-1 (4 × 106 cells) by electroporation. Transfected cells were maintained at 37 °C in DMEM with 10% FCS under 5% CO2 in air for 12 hours. To enable selection of neomycin-resistant clones, transfected cells were maintained at 37 °C in DMEM with 10% FCS containing 500 μg/mL of G 418 (Wako, Osaka, Japan) in SKN or 800 μg/mL in SK-LMS-1. After 14 days, the G418-resistant colonies were harvested.

**DNA extraction and Southern blotting.** High-molecular-weight DNA was prepared from the cultured cells. The cells were incubated for 1 hour at 55 °C in a lysis buffer (10 mM Tris–HCl [pH 8.0], 0.15 M NaCl, and 10 mM ethylenediaminetetraacetic acid [EDTA], 0.1% sodium dodecyl sulfate [SDS], and 100 μg/mL proteinase K [Boehringer Mannheim GmbH, Mannheim, Germany]). Then, 0.5 mL of phenol (water saturated) and 0.5 mL of chloroform were added to the homogenate, with thorough mixing for 20 minutes by inversion. Samples were then centrifuged at 2000g for 10 minutes at room temperature. After centrifugation, the aqueous phase was transferred to a fresh tube. Then, 1 mL of cold 100% ethanol was added, and the DNA was washed in 75% ethanol, dried, and dissolved in 10 mM Tris–HCl (pH 7.4) before being used for Southern blotting. The DNAs were restricted with BamHI (New England Biolabs, Beverly, MA) and fractionated on 0.7% agarose gels. Samples were transferred onto nylon filters (Hybond-N+; Amersham, Buckinghamshire, U.K.), and the filters were probed using a human calponin h1 cDNA labeled with 32P by the random-primer method (BcaBESTTM Labelling kit; Takara Shuzo Co. Ltd., Otsu, Japan). Hybridizations were carried out in 50% formamide, 0.1% SDS, 5x SSPE (525 mM NaCl, 50 mM NaH2PO4, and 5 mM EDTA-Na2 [pH 7.4]), and 5x Denhardt’s solution at 42 °C for 12 hours. Filters were then washed twice in 0.1x standard saline citrate (15 mM NaCl and 1.5 mM Na2 citrate [pH 7.0]) and 0.1x SDS at 55 °C for 1 hour and visualized with the use of a MacBAS system (Fuji Film, Tokyo, Japan).

**RT–PCR analysis.** Total RNA was extracted by the acid guanidine–phenol–chloroform method, as described previously (12,23). After 1 μg of total RNA had been treated with 1 U per 10 μL deoxyribonuclease (DNase) I (Life Technologies Inc., Gaithersburg, MD) at room temperature for 15 minutes, an RT–PCR assay was performed using an RNA PCR Kit (Takara Shuzo Co. Ltd.). The RT step was performed by adding the 1-μg sample of DNase I-treated RNA (see above) to 20 μL of a reaction mixture consisting of 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 5 mM MgCl2, 1 μM deoxynucleoside triphosphate mixture, 1 μM of ribonuclease inhibitor, 0.25 U per μL of avian myeloblastosis virus reverse transcriptase, and 0.125 μM of oligo dT-adaptor primer. The RT reaction was carried out at 42 °C for 30 minutes and stopped by heating at 99 °C for 5 minutes, and the solution was then cooled to 5 °C for 5 minutes. This sequence was carried out on a thermal cycler (Temp Control System PC-700; Astec, Fukuoka, Japan). The amplification step was carried out by adding 80 μL of polymerase chain reaction (PCR) reaction mixture containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2.5 μL per 100 μL of TaKaRa Taq DNA polymerase, and 0.2 μM of two 20–22-mer oligonucleotide primers specific either for the calponin h1 cDNA or for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA. Primers were synthesized to encompass almost the entire C-terminal region of the cDNA sequence of calponin h1 (22) (sense, 5'-AAGGCGG GAA CATCATTGGG T-3' and antisense, 5'-CTCGAA GATCGGC CCTGGT-3'). To confirm the integrity of the RNA, the same amount of cDNA was amplified by PCR using GAPDH primers (sense, 5'-AGCAGACATTTGCACAG CTC-3' and antisense, 5'-TCAGCG TTGGCATGATAC-3') spanning 226 bp between exons 7 and 8. The corresponding cDNA fragments were denatured at 94 °C for 1 minute, annealed at 58 °C for 1 minute, and extended at 72 °C for 2 minutes. After 30 cycles of amplification, the PCR products were analyzed on a 2% agarose gel, and the bands were visualized by ethidium bromide upon exposure to an ultraviolet transilluminator.

**Western blotting.** Cultured cells (calponin h1-transfected cells and vector-only transfected control cells) were lysed in a lysis buffer (50 mM Tris–HCl [pH 8.0], 0.25 M NaCl, 0.5% NP-40, and 1 mM phenylmethylsulfonyl fluoride [Sigma Chemical Co., St. Louis, MO], 1 μg/mL aprotinin [Boehringer Mannheim], 1 μg/mL leupeptin [Boehringer Mannheim], and 20 μg/mL t-1-chlor-3(4-tosylamido)-4-phenyl-2-butanon [Boehringer Mannheim]). The lysates were centrifuged at 13 000g for 20 minutes at 4 °C and the supernatants were stored at −80 °C. Extracts equivalent to 30 μg of total protein were separated by SDS–polyacrylamide gel electrophoresis (10% acrylamide) and transferred onto nitrocellulose membranes (Hybond TM-C super, Amersham). The membranes were blocked in TBST (0.2 mM NaCl, 10 mM Tris [pH 7.4], and 0.2% Tween 20) containing 5% nonfat dry milk and 0.02% NaN3 for 1 hour, then incubated with mouse monoclonal antibodies against calponin h1 (hCP; Sigma Chemical Co.) in TBST containing 5% nonfat dry milk. Membranes were then incubated with sheep anti-mouse immunoglobulin (Amersham) in TBST containing 2% nonfat dry milk. Bound antibody was detected with an enhanced chemiluminescence system (Amersham).

**Immunohistochemistry.** Immunofluorescence analysis of calponin h1 protein and actin was performed on calponin h1-transfected cell lines. Cultured cells in a two-well Lab-Tek Chamber Slide were fixed in cold acetone for 10 minutes. Then, 0.3% hydrogen peroxide was applied to block endogenous peroxide activity, and the cells were incubated with normal goat serum to reduce nonspecific binding. The cells, still on the Lab-Tek Chamber Slide, were then incubated with anti-human calponin h1 monoclonal antibody (hCP; Sigma Chemical Co.) at room temperature for 1 hour, and fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin G was used to obtain green fluorescence from calponin h1. Rhodamine phalloidin (Wako) was used to stain actin to obtain a red fluorescence. All specimens were examined using a Zeiss scanning laser confocal microscope in fluorescence mode (LSM410; Carl Zeiss, Thornwood, NY).

**Morphology of transfected cells.** The transfected leiomyosarcoma cells were observed by daily examination under a phase-contrast microscope (Olympus, Tokyo, Japan).

**Analysis of cell proliferation in cultured cells.** Cell proliferation in cultures of calponin h1-transfected cell lines and vector-only transfected cell lines was analyzed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric method, which is a way to detect enzymes associated with DNA synthesis in dividing cells (24). Transfected cells were plated at a density of 4 × 103 cells/100 μL in a 96-well flat-bottomed tissue-culture plate (Corning Laboratory Sciences Co., NY). The cells were incubated at 37 °C in 5% CO2 in air in DMEM with 10% fetal calf serum. After incubation, an MTT assay was performed. MTT reagent (Research Organics Inc, Cleveland, OH) was diluted to 5 mg/mL in calcium- and magnesium-free Dulbecco’s phosphate-buffered saline (Nissui Pharmaceutical Co., Tokyo, Japan), and this solution was filter sterilized and stored at 4 °C. At designated intervals during the incubation of the transfected cells in the tissue-culture plates, 20 μL of the MTT reagent was added per well, and the plates were reincubated under the prescribed conditions for an additional 4 hours. Then, 100 μL of

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extraction buffer was dispensed per well, and the plates were incubated further until all of the blue crystals were solubilized. The absorbance of the wells was measured by use of an enzyme-linked immunosorbent assay microplate reader (Molecular Devices, Menlo Park, CA) at a wavelength of 570 nm.

**Assay for anchorage-independent and anchorage-dependent growth.** The transfected leiomyosarcoma cells were treated with trypsin, and then approximately 5 × 10⁴ cells were seeded into 0.36% agar (Life Technologies, Inc.) containing DMEM and 10% FCS on top of a bed of 0.72% agar in 35-mm dishes (Costar, Cambridge, MA). Incubation was carried out at 37°C in 5% CO₂ in air. After a 1-week incubation, 1 mL of 0.36% agar was added to avoid drying out. After 21 days, the number of individual colonies larger than 0.05 mm in diameter was determined microscopically at ×100 magnification. The size of the colonies was measured and they were classified according to their diameter, large colonies being 3 mm or more in diameter and small colonies being less than 3 mm in diameter.

**Nude mouse tumorigenicity assay.** The transfected leiomyosarcoma cells were trypsinized, then resuspended in DMEM and 15% FCS at a concentration of 1 × 10⁶ cells/mL. Six-week-old nude mice were given injections of 2 × 10⁴ cells in the flank subcutaneously. Two similar experiments were conducted 8 weeks apart, the only difference between the experiments being the treatment of nude mice—vector-only transfected cells in 12 mice and calponin h1-transfected cells in nine mice; experiment 2—vector-only transfected cells in six mice and calponin h1-transfected cells in six mice). The care and use of these experimental animals were in accordance with institutional guidelines. The mice were observed weekly, and tumor growth was measured. After 2 months, the mice were killed and tumors were excised and fixed in 10% phosphate-buffered formalin, then embedded in paraffin. Sections were prepared for hematoxylin–eosin staining.

**RESULTS**

**Cell Culture and DNA Transfection**

Southern blot analysis of the genomic DNA isolated from SKN and SK-LMS-1 cells transfected with a human calponin h1 expression plasmid after hybridization with a human calponin h1 cDNA probe revealed DNA integration into the genomic DNA. The parental leiomyosarcoma cells and vector-only transfected control cells each showed two bands for the calponin h1 gene, whereas calponin h1-transfected cells showed an additional band in both cell lines (Fig. 1, A). These data indicate that the transfectants were bearing the exogenous calponin h1 gene.

Calponin h1-transfected cells and vector-only transfected cells were analyzed for calponin h1 transcripts by RT–PCR amplification (Fig. 1, B). An intense band was observed at 215 bp for calponin h1 following the RT reaction when RNA extracts from calponin h1-transfected leiomyosarcoma cells were used. In contrast, this band was not obtained from vector-only transfected control leiomyosarcoma cells, although the 226-bp band for GAPDH was obtained for all of these cell lines.

To examine the expression of calponin h1 protein, we analyzed the lysates of calponin h1-transfected and vector-only transfected leiomyosarcoma cells by western blotting. A band of protein at 34 kd for calponin h1 was revealed in calponin h1-transfected cells, but not in vector-only transfected cells (Fig. 1, C), although the 42-kd band for β-actin was obtained for all the cell lines.

**Fig. 1.** Transfection of human leiomyosarcoma cells with calponin h1 complementary DNA. A) Southern blot analysis of DNA. Calponin h1-transfected human leiomyosarcoma cells (lanes 1 and 4), vector-only transfected control leiomyosarcoma cells (lanes 2 and 5), and untransfected parental leiomyosarcoma cells (lanes 3 and 6). The parental leiomyosarcoma cells and vector-only transfected cells each showed two bands for the calponin h1 gene, whereas calponin h1-transfected cells showed an additional band in both cell lines. Lanes 1–3—SK-LMS-1 human leiomyosarcoma cell line; lanes 4–6—SKN human leiomyosarcoma cell line. B) Reverse transcription (RT)–polymerase chain reaction assay for calponin h1. An intense band was observed at 215 base pairs (bp) for calponin h1 only when the RT reaction was performed using RNA extracts from calponin h1-transfected leiomyosarcoma cells (lanes 1 and 4); no such band was observed for vector-only transfected control leiomyosarcoma cells (lanes 2 and 5) or parental leiomyosarcoma cells (lanes 3 and 6). The 226-bp band for glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was obtained for all of the RT reactions (lanes 1–6). Lanes 1–3—SK-LMS-1 cells; lanes 4–6—SKN cells. C) Analysis of calponin h1 protein by western blotting. An intense band of protein at 34 kilodaltons (KD) for calponin h1-transfected cells (lanes 1 and 3) was revealed. In contrast, 34-KD bands were not observed for vector-only transfected cells (lanes 2 and 4). A band (42 KD) for β-actin was observed in the protein extracts from all of the cell lines. Lanes 1 and 2—SKN cells; lanes 3 and 4—SK-LMS-1 cells. D) Double-staining immunofluorescence analysis for calponin h1 and actin in transfected cells, using a Zeiss scanning laser confocal microscope. Anti-calponin h1 antibody was conjugated with fluorescein isothiocyanate and used to obtain a green fluorescence. Rhodamine phalloidin was used to stain actin to obtain a red fluorescence. Vector-only transfected control leiomyosarcoma cells (a and e) and calponin h1-transfected leiomyosarcoma cells (b and d). Positive staining for calponin h1 was observed in calponin h1-transfected leiomyosarcoma cells (b and d). a and b—SKN cells; c and d—SK-LMS-1 cells (×630). See text for more methodologic detail.
Immunofluorescence staining of the transfected SKN and SK-LMS-1 cells was performed using hCP, a species-specific monoclonal antibody against calponin h1. Specific staining for calponin h1 was observed as a green color in the cytoplasm, and actin was detected as a red color. Immunofluorescence analysis revealed diffuse staining of calponin h1 in the cytoplasm of calponin h1-transfected leiomyosarcoma cells. In contrast, the expression of calponin h1 was faint or absent in vector-only transfected leiomyosarcoma cells (Fig. 1, D). These results confirmed that expression of calponin h1 protein was present in calponin h1-transfected leiomyosarcoma cells, but that little or no expression of calponin h1 protein was present in vector-only transfected control leiomyosarcoma cells.

Morphology of Transfected Cells

To determine whether morphologic changes are induced by calponin h1 transfection in leiomyosarcomas, we made observations by phase-contrast microscopy. Calponin h1 produced a situation in which elongated spindle-shaped cells came to lie with their major axes in parallel, and there was a “hills and valleys” growth pattern similar to that seen in cultures of normal myometrial SMCs (Fig. 2). In contrast, vector-only transfected control leiomyosarcoma cells grew in an irregular fashion, as if there was no contact inhibition.

Analysis of Cell Proliferation in Cultured Cells

The effect of calponin h1 on cell growth was analyzed. An MTT assay showed that the number of cultured cells per 96-well plate was reduced following calponin h1 transfection of leiomyosarcoma cells (Fig. 3). The proliferation of calponin h1-transfected leiomyosarcoma cells was reduced to 70% ($P = .017$) of control in SKN and to 69% ($P = .023$) in SK-LMS-1. The cell numbers indicated significant inhibition of cell proliferation by calponin h1 transfection in both SKN and SK-LMS-1.

Assay for Anchorage-Independent and Anchorage-Dependent Growth

Normal cells must attach to a substratum to grow, whereas tumorigenic cells need not and can therefore form colonies on soft agar plates. To determine the effect of calponin h1 expression on colony formation by SKN cells on soft agar, we examined the anchorage-independent growth. Calponin h1-transfected cells showed much higher anchorage-dependent growth than vector-only transfected control cells. The mean number of large colonies was 10.4 (95% confidence interval [CI] = 9.02–12.28) for vector-only transfected cells and 1.5 (95% CI = 0.68–2.27) for calponin h1-transfected cells, a statistically significant reduction to about one seventh of control values ($P = .004$).

Nude Mouse Tumorigenicity Assay

To further examine the tumor-suppressor activity of calponin h1 in leiomyosarcoma tumor development, we tested whether increased expression of calponin h1 protein in leiomyosarcoma cells might reduce tumor formation when these cells were implanted into nude mice. The SK-LMS-1 cell line was chosen because it causes tumor formation when injected into nude mice. While vector-only transfected control cells did indeed induce tumors (in nine of 12 mice and in four of six mice, respectively, in two similar experiments) within 1 month of their injection, no tumors (in none of nine mice and in none of six mice, respectively) had developed within 1 month in animals injected with calponin h1-transfected cells. Two months after the injection of calponin h1-transfected cells, small tumors had developed in two of nine mice in one experiment (Fig. 4, A), while no tumors had developed in any of the six mice in a second experimental series. The nude-mouse tumorigenicity of calponin h1-transfected SK-LMS-1 cells was significantly reduced after the first month ($P < .0001$) and after 2 months ($P = .001$). In terms of pathology, the tumors produced by vector-only transfected leiomyosarcoma cells contained cells that were arranged irregularly, and they were considered to have a malignant phenotype (Fig. 4, B-a). In contrast, the tumors induced by calponin h1-transfected cells were composed of elongated spindle-shaped cells with their major axes lined up in parallel (Fig. 4, B-b). These results suggested that the tumorigenicity of calponin h1-transfected cells is much lower than that of the vector-only transfected controls.

DISCUSSION

Our examination revealed that the arrangement of human calponin h1-transfected leiomyosarcoma cells resembles the morphology seen in cultured normal myometrial SMC (in which elongated, spindle-shaped cells usually grow with their major axes in parallel and exhibit a growth pattern of hills and valleys), whereas leiomyosarcoma cells and vector-only transfected leiomyosarcoma cells grow in an irregular fashion, as if there were no contact inhibition. In other words, the re-expression of the calponin h1 protein seems to induce in leiomyosarcoma cells a behavior resembling that of...
normal SMC in vitro. In addition, the proliferation of calponin h1-transfected leiomyosarcoma cells was inhibited by 30% compared with control SKN cells and by 31% compared with control in SK-LMS-1 cells. In a colony formation assay, the number of large colonies was found to be reduced to about one seventh of control levels by calponin h1 transfection in SKN cells. In addition, the tumorigenicity in nude mouse of calponin h1-transfected SK-LMS-1 cells was found to be reduced. In short, following human calponin h1 transfection, the proliferation, colony formation, and tumorigenicity of leiomyosarcoma cells were all reduced and they acquired the behavior typical of normal SMC, which might be an indication of differentiation.

A decreased level of calponin h1 expression has been found in hyperplastic and dedifferentiated SMC (26). The calponin h1 gene is expressed in resting rat aortic SMC, but its expression rapidly decreases when growth-arrested cells re-enter the G1 phase of the cell cycle and proliferate (18). Moreover, adenovirus-mediated transfer of the smooth muscle calponin gene inhibits proliferation in SMC and fibroblasts (27). Because cell shape controls cell proliferation (28) and because cell attachment and spreading are essential steps for cells to traverse through the G1 phase of the cell cycle (29), our present observations on cell morphology and proliferation following calponin h1 transfection suggest that calponin h1 may inhibit the signaling pathways concerned with the control of cell attachment, cell shape, and spreading—processes in which the cytoskeleton is intimately involved. This being so, we suggest that regulation of calponin h1 expression can modulate cell proliferation activity.

Calponin h1 most likely exerts its biologic effects through interactions with other cellular molecules that transduce signals from outside the cell. Calponin can promote actin filament stability in vitro by inhibiting depolymerization (30). In addition, calponin inhibits the magnesium-dependent adenosine triphosphatase activity of actomyosin by binding to actin, and this inhibition is reversed by phosphorylation of calponin or interaction of calponin with Ca2+-binding proteins such as calmodulin (12,31). The potentially important action of calponin h1 in leiomyosarcomas may be related to its stabilizing effect on the thin filaments, which are built of F-actin associated with tropomyosin and troponin, an effect that might result in increased resistance to the cytoskeletal rearrangements stimulated by exposure to mitogens. Hence, calponin h1 may also depress the rate of cell proliferation by inhibiting actomyosin-dependent processes.

It is increasingly clear that in many instances features of the cellular phenotype in vascular SMC are modulated locally by cytokines or growth factors and by extracellular matrix components rather than by genetically irreversible steps (32). However, the control of cell proliferation and phenotypic features has not yet been clearly explained in uterine SMC. It has been reported that the expression of muscle-specific markers is reduced in malignant smooth muscle neoplasms (33–36). In addition, repression of smooth muscle α-actin gene expression has been reported in ras-transformed cells (37), and numerous transformed cell lines have been found to display a loss of intracellular actin fragments, a condition that is associated with substrate independence and in vivo tumorigenicity (38). This suggests that repression of cytoskeletal genes is critical to the maintenance of the transformed phenotype.
Previously (2), we demonstrated immunohistochemically that, while calponin h1 is expressed in normal myometrium and all leiomyomas, its expression is markedly weaker in leiomyosarcoma. In the present report, we further demonstrate that, although leiomyosarcomas possess the calponin h1 gene, the transcription of the calponin h1 gene is most likely suppressed and that re-expression of the human calponin h1 protein suppresses cell proliferation and tumorigenicity in leiomyosarcoma cells. The molecular mechanisms by which the expression of calponin h1 is reduced in leiomyosarcoma are not clear. Since it has been reported that methylation of the promoter region may block transcription, and that although leiomyosarcomas possess the calponin h1 gene, its expression is markedly weaker in leiomyosarcoma, we further demonstrate in the present report that methylation of the promoter region may block transcription.

The elucidation of the mechanism by which human calponin h1 expression is regulated may provide a great deal of information about the transformation of cellular phenotypes, the control of cell proliferation activity, and the pathogenesis of uterine smooth muscle neoplasia at the molecular level.

In conclusion, we have shown that re-expression of the human calponin h1 protein suppresses cell proliferation and tumorigenicity in leiomyosarcoma cells, suggesting that calponin h1 may have a possible role as a tumor suppressor in human uterine leiomyosarcoma.

REFERENCES

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NOTES


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