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SUPPRESSION OF PERITONEAL DISSEMINATION THROUGH PROTECTING MESOTHELIAL CELLS FROM RETRACTION BY CANCER CELLS

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In a previous study, we demonstrated that calponin h1 suppressed tumor growth of transformed cells and that the peritonitis carcinomatosa induced by mouse B16-F10 melanoma (F10) cells was more extensive in calponin h1-deficient ($CN^{-/-}$) mice with fragility of mesothelial (MS) cells than in their calponin h1-wild ($CN^{+/+}$) counterparts. In our study, we assessed the therapeutic effect of calponin h1 on peritoneal dissemination. F10 cells were overlaid on the cultured $CN^{+/+}$ or $CN^{-/-}$ MS cells and the effect of calponin h1 on retraction of MS cells was evaluated. Then, an adenoviral vector with the calponin h1 gene (AdGFP-CN) inserted was constructed and was applied to $CN^{-/-}$ MS cells or $CN^{-/-}$ mouse peritoneum to investigate its suppressive effect on the peritoneal dissemination caused by F10 cells. Greater retraction and invasion of F10 cells were observed in $CN^{-/-}$ MS than in $CN^{+/+}$ cells *in vitro*, while down-regulation of calponin h1 was observed in $CN^{+/+}$ MS cells prior to the invasion of F10 cells. Infecting $CN^{-/-}$ MS cells with AdGFP-CN prevented their retraction and the invasion of F10 cells. Peritoneal dissemination was prominently suppressed in AdGFP-CN-infected $CN^{-/-}$ mice, and the survival of those mice was significantly prolonged. Thus, calponin h1 functioned to protect host MS cells from the invasion of F10 cells.

Key words: *calponin h1; calponin h1-deficient mice; peritoneal dissemination; gene therapy; adenovirus*

Peritoneal dissemination is one of the major patterns of postoperative recurrence and as a result, a major cause of death in advanced gastric cancer or pancreatic ductal adenocarcinoma.[1] Despite improvements in chemotherapy and surgical techniques, prognoses for peritoneal dissemination remain pessimistic;[2] thus, development of a new therapy and/or protection for peritoneal dissemination has been the subject of intensive research.

The mesothelial (MS) cell monolayer on the peritoneum has been reported to prevent infiltration of cancer cells into the peritoneum,[3] while peritoneal fibrosis induced by gastric cancer cells may provide a congenial environment for peritoneal dissemination.[4] These reports led us to hypothesize that strengthening the capability of MS cells to prevent the invasion of cancer cells might result in an effective treatment for peritoneal dissemination.

Calponin h1 is a 34 kDa protein, originally identified in chicken gizzard,[5] and is reported to stabilize actin filaments,[6] inhibit the tumor growth[7] and increase cell adhesion.[8] Three calponin isoforms, calponin h1, h2 and acidic calponin, have been identified and characterized.[9–11] Calponin h1 is mainly expressed in the smooth muscle cells, while we observed its expression also in peritoneal MS cells.[12] Calponin h2 is a nonmuscle-type protein and acidic calponin is mainly found in the brain. Differences in the biological functions of these isoforms constitute an intriguing issue but remain to be clarified,[13] although recently it has been reported that calponin h1 is a novel substrate of Rho kinase,[14] and is involved in the MAPK,[15] PKC[16] and ERK signaling pathways.[17]

We have previously reported on the down-regulation of smooth muscle α-actin (αSMA) in the blood vessels of malignant tumors.[18–20] We also observed that melanoma cells released PDGF-BB, a product of the *sis* oncogene, which suppressed αSMA,[20] and that the down-regulation of calponin h1, which is also induced by PDGF-BB[21] as seen in αSMA, was greater than that of αSMA.[12] Assuming that the reduction in calponin h1 leads to the fragility of blood vessels, we generated calponin h1-deleted mice ($CN^{-/-}$) and observed that the structure of blood vessels and peritoneum was indeed fragile in these mice. This fragility resulted in enhancement of both the hematogenous metastasis and the peritoneal dissemination of mouse B16-F10 melanoma (F10) cells.[12]

In our study, we observed that prior to their invasion, F10 cells suppressed calponin h1 expression in $CN^{+/+}$ MS cells. We also examined whether calponin h1 in $CN^{-/-}$ mice strengthens the function of the peritoneum as a barrier against the invasion of F10 cells.

MATERIAL AND METHODS

Animals and cells

$CN^{-/-}$ mouse was generated as previously described.[22] Mesothelial (MS) cells were obtained from the mouse mesentery after sacrifice. All animal handling procedures were in accordance with a protocol approved by the Ethics Committee of Shinshu University School of Medicine. The mesenteries were briefly washed in phosphate-buffered saline (PBS) (Nissui, Tokyo, Japan) and treated with 0.25% trypsin (DIFCO, Detroit, MI). MS cells from the mesenteries were obtained by filtration (150MC, Ikedarika Co., Tokyo, Japan) and cultured to confluency in a 60 mm dish containing Dulbecco's modified eagle medium (DMEM) (Wako, Osaka, Japan), which included 10% fetal bovine serum (FBS) (GIBCO-BRL, Grand island, NY) and 1% streptomycin (GIBCO). Mouse B16-F10 melanoma (F10) cells and a human kidney-derived cell line, known as 293 cells (TaKaRa Co., Ltd, Ootsu, Japan), were cultured in the same medium.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis

The method used has been described previously.[8] In brief, total RNA was extracted from $CN^{+/+}$ or $CN^{-/-}$ MS cells with the acid guanidium-phenol-chroloform method using ISOGEN (Nippon Gene, Tokyo, Japan). RT-PCR was performed with the aid of an RNA PCR Kit (TaKaRa). The primers for mouse calponin h1 were sense 5′-GTCTGTGTCATCTGCACCTC-3′ and anti-sense 5′-TCCCGTCGCAGGAATGGGGC-3′; for mouse calponin, h2 sense 5′- CCCTCTGCCGGTCCCGCTGG-3′ and anti-sense 5′-CAAACTGATGTGAAGAGAT-3′; and for mouse acidic calponin, sense 5′- AACAGCCAGACCCACTTCAAC-3′ and anti-

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sense 5′- TCGGGGTATTCTGCTGATAATC-3′.[22] Primers for glyceraldehydes-3-phosphate dehydrogenase (GAPDH) were sense 5′- AAGGGCGCAACATCATTGGGCT-3′ and anti-sense 5′- TCACAGTTGCCATGTAGACC-3′.

Immunohistochemistry for calponin h1

MS cells obtained from $CN^{-/-}$ and $CN^{+/+}$ mice were cultured separately on micro glass slides (Matsunami, Inc., Osaka, Japan). After rinsing with PBS, the cells on each glass slide were fixed with 50% ethanol for 5 min, 75% ethanol for 5 min and 100% ethanol for 5 min. After another rinsing with PBS, the cells were treated with 0.1% Triton X-100 (Wako) in PBS and 1% albumin bovine in PBS, both for 10 min. Rabbit anti-mouse calponin antibody[22] was applied to fixed MS cells on glass slides for 3 hr at room temperature. After washing with PBS, goat anti-rabbit fulorescein isothiocynate (FITC)-conjugated antibody (DAKO, Kyoto, Japan) was applied to the preparations as a secondary antibody. The specimens were then examined with a fluorescent microscope (Axiovert S100, Zeiss, Thornwood, NY).

Examination of MS cells overlaid with F10 cells in vitro

MS cells obtained from euthanized $CN^{+/+}$ or $CN^{-/-}$ mice were cultured to confluency on a 24-well tissue culture plate (FALCON, Franklin Lakes, NJ). F10 cells (3×10^4) were overlaid on the MS cells in each well. The retraction of MS cells and the invasion of F10 cells were observed with a phase-contrast microscope (Nikon, Tokyo, Japan) 3, 6 and 12 hr after the overlaying .[23]

Construction of recombinant adenovirus

We constructed a recombinant adenovirus infected with the calponin h1-green fluorescent protein (GFP) fusion gene (AdGFP-CN) and with the GFP gene (AdGFP) for the control. Human calponin h1 gene was inserted into pEGFP-C2 (Clontech, Palo Alto, CA) to produce the GFP-human calponin h1 fusion gene. This was followed by subcloning of the fusion gene under the transcriptional control of CAG promoter/enhancer (cytomegalovirus enhancer, chicken β-actin promoter, rabbit β-globin poly A signal) present in cosmid vector pAxCAwt (Adenovirus expression kit :TaKaRa). The recombinant cosmid vector was packaged with Gigapack XL (Stratagene, CA)[24] and transformed to DH5α *E. coli* (*recA*−). The cloned cosmid vector and adenovirus genome DNA tagged with the terminal protein complex (DNA-TPC), which has a high recombination efficiency, were then cotransfected to the 293 cells cultured to confluency in a 60 mm dish with the aid of a mammalian transfection kit (Stratagene). Recombination took place in the 293 cells and the recombinant was amplified with the E1A gene in the 293 cells.[25] To obtain high titer viruses, 293 cells were infected 4 times with the prepared viruses. The titer of these viruses was then adjusted to 4×10^9 plaque forming units (PFU)/ml. Thus $CN^{-/-}$ MS cells cultured on micro glass slides were infected with prepared AdGFP or AdGFP-CN. Two days after the infection, the treated MS cells were observed by fluorescent microscopy with a FITC filter to determine the GFP distribution in each of the infected cells. $CN^{-/-}$ cultured MS cells or peritoneum of $CN^{-/-}$ mice were infected with AdGFP or AdGFP-CN, extracted and lysed with SDS buffer for SDS-PAGE analysis.[7] Samples were electrophoresed and applied to Western blot analysis using mouse anti-human calponin h1 antibody (DACO).

Assay of biological phenotype of the adenovirus-infected $CN^{-/-}$ MS cells in vitro *and* in vivo

AdGFP or AdGFP-CN (titer: 4×10^8 PFU/0.1 ml) was applied to $CN^{-/-}$ MS cells cultured to confluence in a 24-well plate. Two days after the infection, 3×10^4/0.5 ml of F10 cell suspension was overlaid on the MS cell layer, and 6 hr later, retraction or disruption of the infected MS cells by the F10 cells was evaluated by means of phase contrast microscopy (Nikon, Tokyo, Japan).

For the *in vivo* experiments, AdGFP-CN (titer: $8\times10_9$ PFU/2 ml) was i.p. injected into $CN^{-/-}$ mice (fluorescence microscopy confirmed that the expression of GFP in almost all of the MS cells was maintained for at least 3 days after the injection of the viruses). Two days after the preinjection of the viruses, 5×10^5 F10 cells were administered i.p., together with the adenovirus, AdGFP-CN or AdGFP, which was injected every 3 days. Fourteen days later, the mice were sacrificed and the extent of peritoneal dissemination in each of the groups was examined. Survival analysis was performed following the same protocol as described above.

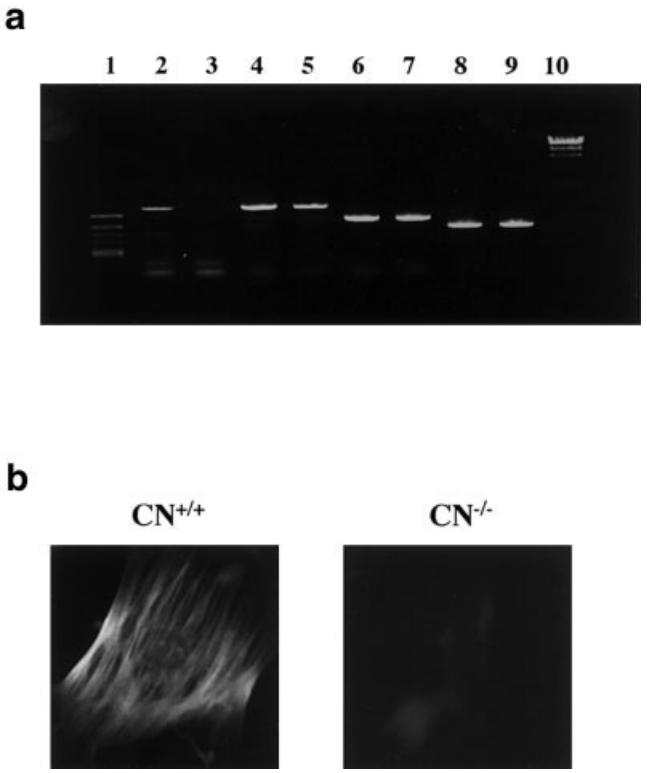

FIGURE 1 – Calponin h1 expression in MS cells. (**a**) RT-PCR assay for calponin isoforms in $CN^{+/+}$ and $CN^{-/-}$ MS cells. A band is observed at 1295 base pairs (bp) for calponin h1 in $CN^{+/+}$ MS cells (lane 2) but not in $CN^{-/-}$ MS cells (lane 3). Bands at 1312bp for calponin h2 (lanes 4,5) at 924bp for acidic calponin (lanes 6,7) and at 781bp for GAPDH (lanes 8,9) are observed in both $CN^{+/+}$ and $CN^{-/-}$ MS cells. Lanes 1 and 10 are markers. (*b*) Immunofluorescence staining of calponin h1 in $CN^{+/+}$ and $CN^{-/-}$ MS cells. Rabbit anti-mouse calponin h1 antibody was conjugated with FITC to obtain green fluorescence.

Statistical analysis

Statistical analysis was performed using StatView 5.0 software (SUS Institute, Inc., Berkley, CA). Survival curves were analyzed with the Kaplan-Meier method and difference between curves was assessed according to the log-rank test. The difference was considered to be significant at $p < 0.05$.

RESULTS

Detection of Calponin h1 in cultured MS cells

MS cells were obtained from both $CN^{+/+}$ and $CN^{-/-}$ mouse peritoneum and cultured to confluency. These cells exhibited a typical cobblestone-like morphology which appeared to be much the same for $CN^{+/+}$ and $CN^{-/-}$ MS cells. RT-PCR analysis of the mRNA of $CN^{+/+}$ and $CN^{-/-}$ MS cells detected expression of calponin h1 only in cultured $CN^{+/+}$ MS cells but not in cultured $CN^{-/-}$ cells. Quantities of Calponin h2 and acidic calponin were equal for $CN^{+/+}$ and $CN^{-/-}$ MS cells (Fig. 1*a*). Immunochemical analysis using rabbit anti-mouse calponin antibody identified cal-

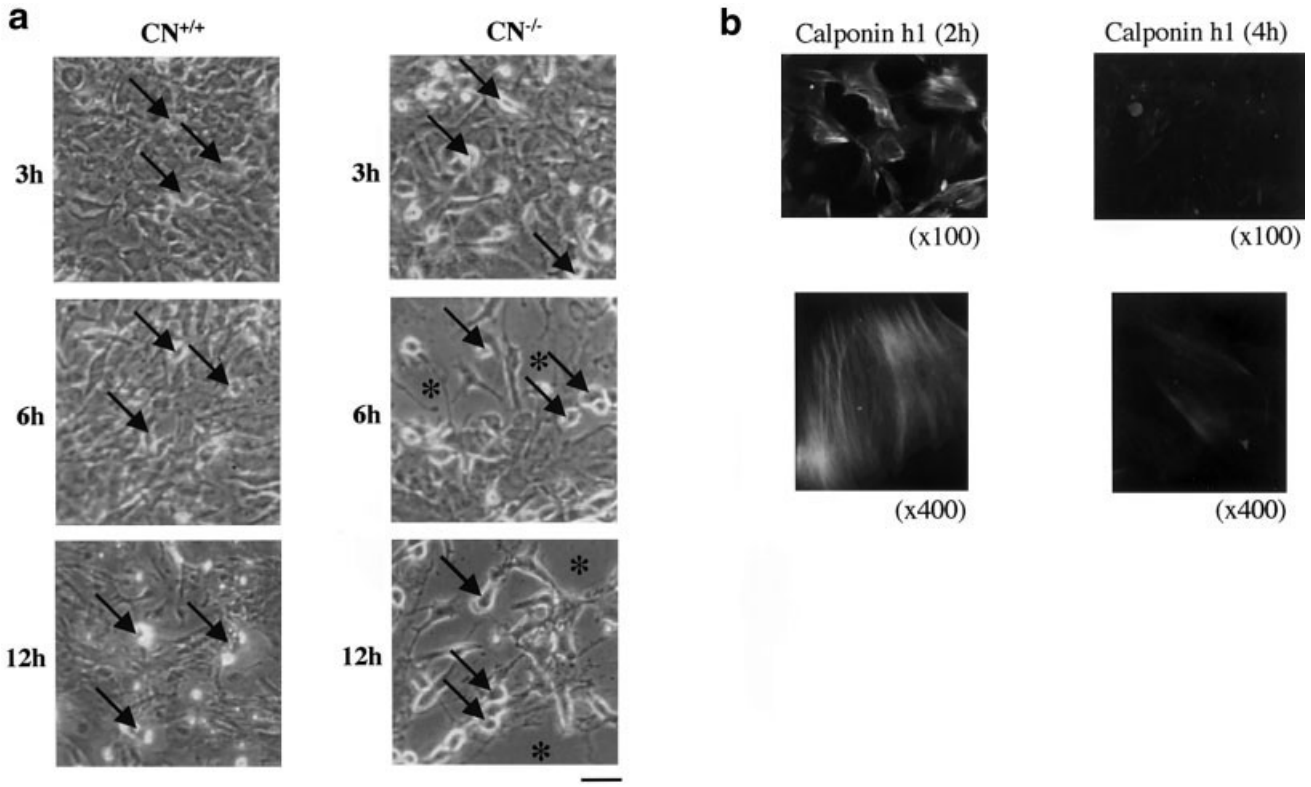

FIGURE 2 – Morphological changes of MS cells as a result of overlaying of F10 cells. (*a*) Retraction and/or disruption analysis of MS cells by overlaying of F10 cells *in vitro*. F10 cells were overlaid on $CN^{+/+}$ or $CN^{-/-}$ MS cells cultured to confluence. Three, 6 and 12 hours after the overlaying, the extent of retraction and/or disruption of MS cells were compared. Arrows indicate the representative F10 cells which appear bright on the surface of MS cells. Stars indicate the exposed substrate after the retraction caused by F10 cells. Scale bar: 6 μm. (*b*) Successive changes in calponin h1 expression after overlaying of F10 cells on cultured $CN^{+/+}$ MS cells. F10 cells were overlaid on $CN^{+/+}$ MS cells and the expression of calponin h1 was immunohistochemically evaluated 2 or 4 hr later.

ponin h1 together with actin filaments in $CN^{+/+}$ MS cells, which is the typical localization of calponin h1 seen in smooth muscle cells. No expression of calponin h1 was detected in $CN^{-/-}$ cells (Fig. 1*b*).

Retraction and/or disruption of MS cells caused by overlaying F10 cells

Three hours after the overlaying of F10 cells, almost all of the F10 cells were only observed on the MS cell layer in both $CN^{+/+}$ and $CN^{-/-}$ (Fig. 2*a*). Six hours later, however, retraction of the $CN^{-/-}$ MS cells occurred (stars), followed by invasion by F10 cells into the $CN^{-/-}$ MS cell layer. In contrast, the $CN^{+/+}$ MS cell monolayer remained almost intact and F10 cells were still seen to adhere only to the surface of the MS cell layer. Twelve hours after the overlaying, severe disruption of the $CN^{-/-}$ MS cell layer and detachment of the MS cells from the bottom of the plate were observed. The intercellular adhesion of the $CN^{+/+}$ MS cells appeared to be slightly diminished but was still relatively strong compared to that of the $CN^{-/-}$ MS cells, and hardly any invasion of F10 cells had occurred, even as late as 12 hr after the overlaying of F10 cells. Complete retraction of the $CN^{+/+}$ MS cell layer was observed 24 he after the overlaying (data not shown). Immunohistochemical examination of the $CN^{+/+}$ MS cells exhibited expression of calponin h1 (Fig. 2*b*). Four hours after the overlaying, the expression of calponin h1 was downregulated (top right Fig. 2*b*) compared to that 2 hr after the overlaying (top left Fig. 2*b*). This down-regulation was also observed by the overlaying of the supernatant of F10 cells (data not shown). While calponin h1 localization along the intact actin fibril structures as seen in the $CN^{+/+}$ MS cells (Fig. 1*b*) was examined in high magnification within 2 hours after the overlaying of F10 cells (bottom left Fig. 2*b*), this was not seen in the cells 4 hr after the overlaying (bottom right Fig. 2*b*).

Infection of AdGFP-CN into $CN^{-/-}$ MS cells

High-titer AdGFP and AdGFP-CN were generated in the 293 cells (Fig. 3*a*). $CN^{-/-}$ MS cells cultured on micro glass slides were then infected with these viruses and the expression of GFP was observed with fluorescent microscopy. The fluorescence image of AdGFP-infected $CN^{-/-}$ MS cells was diffuse (Fig. 3*b*). In contrast, that of AdGFP-CN-infected $CN^{-/-}$ MS cells exhibited the typical image of calponin h1 as seen in the $CN^{+/+}$ MS cells in Figure 1*b*: localization of calponin h1 along the actin filaments (Fig. 3*b*). Western blot confirmed the expression of human calponin h1 in AdGFP-CN-infected $CN^{-/-}$ MS cells and the absence of calponin h1 expression in AdGFP-infected cells (Fig. 3*c*).

AdGFP-CN infection into $CN^{-/-}$ MS cells and their acquisition of resistance against invasion of F10 cells in vitro

To examine the effects of calponin h1 on the retraction of MS cells, $CN^{-/-}$ MS cells that were cultured to confluence were infected with AdGFP or AdGFP-CN. When F10 cells were overlaid on the MS cells, the AdGFP-infected $CN^{-/-}$ MS cells proved to be fragile and retraction was observed in almost all of them 6 hr after the overlaying (Fig. 4), as was also seen in $CN^{-/-}$ MS cells after the overlaying of F10 cells (Fig. 2*a*). In contrast, most of the AdGFP-CN-infected $CN^{-/-}$ MS cells appeared still to have kept their morphology intact, and F10 cells adhered to the surface of the cell layer; that is, there was little retraction and/or disruption caused by the F10 cells and no invasion by F10 cells was observed at this time. These phenomena were very similar to those seen in $CN^{+/+}$ MS cells 6 hr after the overlaying of F10 cells (Fig. 2*a*).

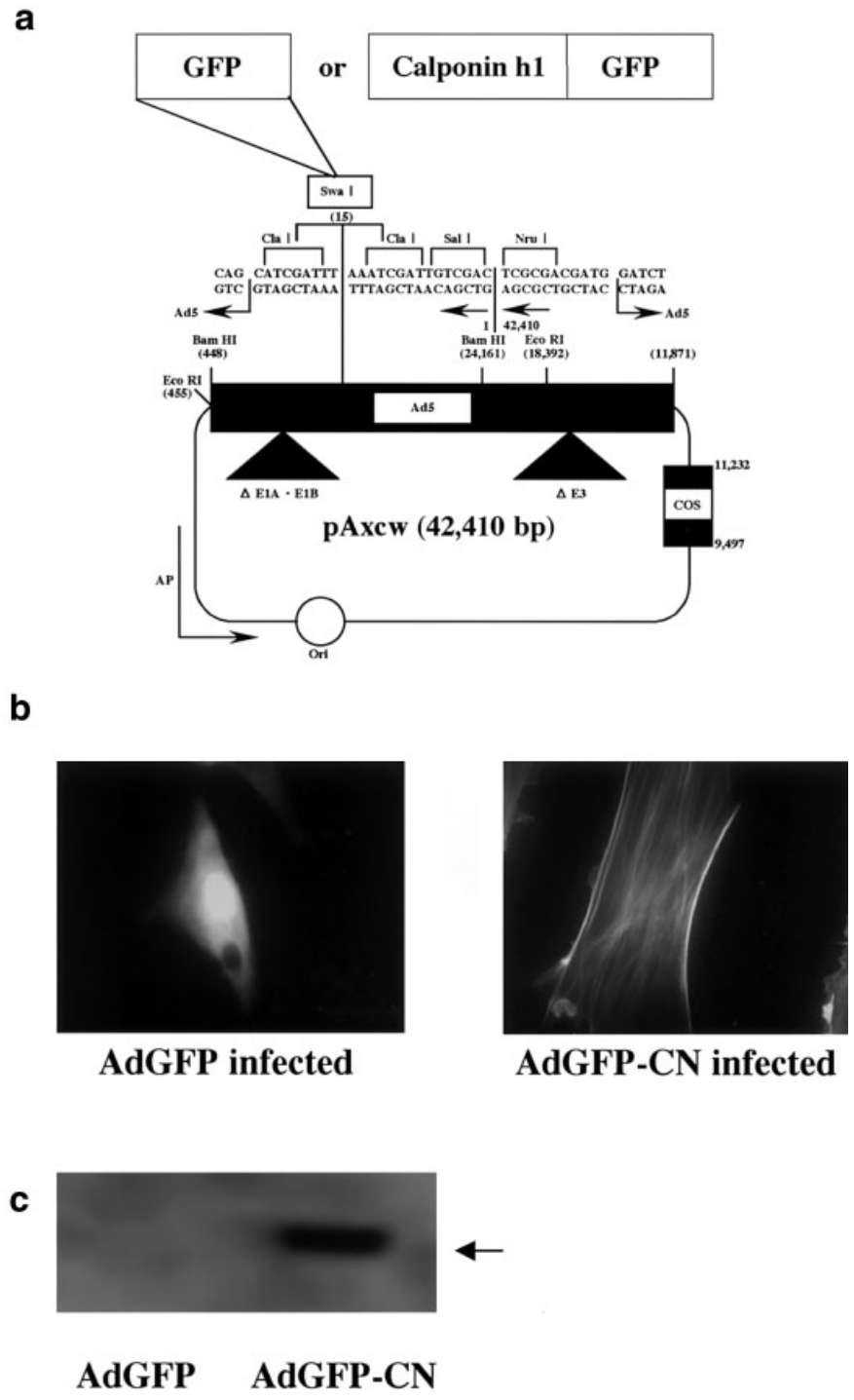

FIGURE 3 – Construction of calponin h1 recombinant adenovirus vector and the expression of calponin h1 in the adenovirus infected $CN^{-/-}$ MS cells. (*a*) Adenovirus-carrying GFP gene (control and AdGFP) and GFP-calponin h1 fusion gene (AdGFP-CN) were constructed as detailed in Material and Methods. (*b*) $CN^{-/-}$ MS cells were infected with AdGFP or AdGFP-CN and the expression of GFP was observed with a fluorescent microscope. (*c*) Western blot for calponin h1 of cultured $CN^{-/-}$ MS cells infected with AdGFP or AdGFP-CN. Arrow indicates fusion protein of GFP and calponin h1 (61 kDa).

In vivo *inhibition of peritoneal dissemination by i.p. AdGFP-CN infection*

The next point to be examined was whether infection of AdGFP-CN can protect $CN^{-/-}$ mice against the peritoneal dissemination caused by F10 cells. The AdGFP- or AdGFP-CN-treated mice were sacrificed, and the extent of peritoneal dissemination was assessed 14 days after the i.p. injection of F10 cells. Expression of human calponin h1 in AdGFP-CN-treated mouse peritoneum was confirmed by Western blot (Fig. 5*a*).

Fewer nodules on the mesentery, fewer ascites and fewer instances of metastasis to other organs were observed in AdGFP-CN than in AdGFP-treated $CN^{-/-}$ mice (Fig. 5*b*). As an example of typical peritoneal dissemination, many nodules on the mesentery, metastases to the liver, kidney, pancreas and spleen, as well as adhesion of the peritoneum to organs, such as gut and stomach, were observed in AdGFP-infected $CN^{-/-}$ mice. All of the AdGFP-treated mice had died of peritoneal dissemination by day 20 after the i.p. injection of F10 cells, while only 1 AdGFP-CN-treated mouse died during the same period. Thus, survival of AdGFP-CN-treated mice was significantly prolonged compared to that of AdGFP-treated mice (Fig. 5*c*).

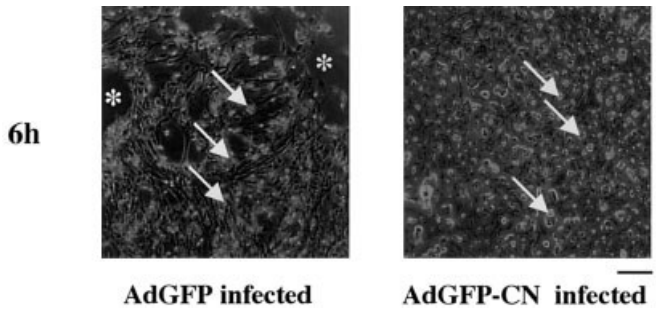

FIGURE 4 – The protective effect of calponin h1 on retraction in $CN^{-/-}$ MS cells caused by F10 cells *in vitro*. F10 cells were overlaid on AdGFP- or AdGFP-CN-infected $CN^{-/-}$ MS cells cultured to confluence. The images were obtained 6 hr after the overlaying of F10 cells. Arrows indicate the representative F10 cells and stars indicate the exposed substrate after the retraction as seen in Figure 2*a*. Scale bar: 3 μm.

DISCUSSION

In a previous report, we described the fragility of blood vessels in $CN^{-/-}$ mice, *i.e.*, weak adhesion of the endothelial cells of the femoral artery to the elastic fiber, rapid fluorescein leakage from the blood vessels of the retina and significant metastasis of F10 cells to the lung. As for the peritoneum, we found that MS cells had shrunken or retracted and that peritonitis carcinomatosa was more severe in $CN^{-/-}$ than in $CN^{+/+}$ mice.[12].. These findings led us to speculate that the deficiency of calponin h1 affects the structural integrity of the peritoneum and intercellular adhesion of MS cells, thus facilitating the extravasation of cancer cells.

In the study presented here, we found that 1) down-regulation of calponin h1 in $CN^{+/+}$ MS cells occurred prior to the invasion of F10 cells, 2) the invasion by F10 cells of the $CN^{-/-}$ MS cell layer was more prominent than that of its $CN^{+/+}$ counterpart and 3) the fragility of $CN^{-/-}$ MS cells was prevented by calponin h1 gene transfer, resulting in lessening of the F10 cell invasion. In *in vivo* experiments, the transfer of the calponin h1 gene to the peritoneal cavity of $CN^{-/-}$ mice resulted in suppression of the invasion of F10 cells into the peritoneum. These results indicate that the interaction of MS cells with cancer cells results in the down-

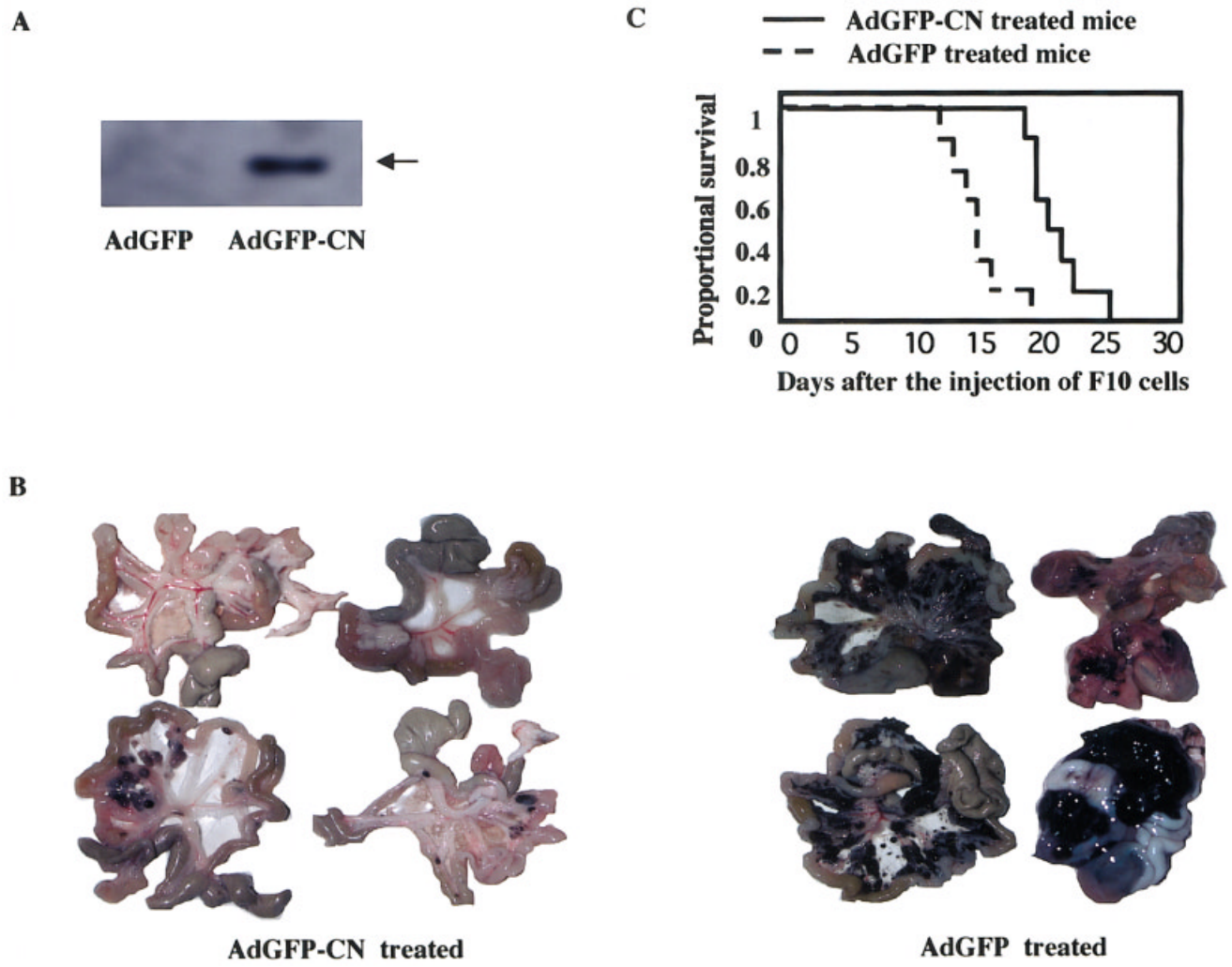

FIGURE 5 – Prevention of peritoneal dissemination of F10 cells by the expression of calponin h1 using adenovirus vector. (*a*) Expression of human calponin h1 in AdGFP-CN-treated mouse peritoneum detected by Western blot. An arrow indicates fusion protein of GFP and calponin h1 (61 kDa). (*b*) F10 cells were injected into the peritoneal cavity of $CN^{-/-}$ mice infected with AdGFP or AdGFP-CN. Virus infection was performed every 3 days. Fourteen days after the i.p. injection of F10 cells, mice were sacrificed and the extent of peritoneal dissemination was examined. (*c*) Survival curve of (n=7) AdGFP- or AdGFP-CN-treated mice (n=7) ($p < 0.05$).

regulation of calponin h1, which leads to the subsequent weakening of the integrity of the MS cell layer. Moreover, the inhibition of the down-regulation of calponin h1 induced by cancer cells is important for protection against peritonitis carcinomatosa, which very often occurs after surgery for gastric cancer, ovarian cancer or pancreatic adenocarcinoma. The precise mechanism causing the down-regulation of calponin h1 by F10 cells is unknown, but several growth factors/cytokines such as platelet-derived growth factors (PDGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF) and transforming growth factors (TGFβs), which are reported to induce dedifferentiation of smooth muscle cells, decreased the calponin expression.[21] On the other hand, melanoma has been shown to secrete PDGF[20,26] and to characteristically express TGFβs and/or bFGF.[27] Taking these findings together, the down-regulation of calponin h1 in MS cells caused by F10 cells might be intermediated by factors, secreted from F10 cells, like PDGF, TGFβs and bFGF.

In our previous study,[8] slightly higher levels of integrin α5β1 were detected on calponin h1 transfected HT1080 cells compared to vector only transfected ones. Activated integrin by anti-β1 antibody, 12G10, is reported to induce cell-cell adhesion besides cell-substrate adhesion through organization of the cytoskeleton, F-actin polymerization.[28] Therefore calponin h1 is considered to be involved in cell-cell adhesion through integrin system. Calponin reportedly accelerates polymerization and inhibits depolymerization of actin,[6] thus increasing resistance of the actin cytoskeleton to antagonists in normal cells.[29] Furthermore, the level of actin expression was reduced in $CN^{-/-}$ mice compared to that in $CN^{+/+}$ mice.[30] We observed that the actin filaments were thinner in $CN^{-/-}$ MS than in $CN^{+/+}$ cells (data not shown). It is reported that cell-cell adhesion *via* the cadherin system is intimately related with actin polymerization.[31] Thus, actin organization caused by calponin h1 also induces intercellular adhesion. Considering these findings together, the weakened intercellular adhesion induced by the deficient of calponin may promote the fragility of MS cells through reduced actin organization. In addition, we recently found that when F10 cells were i.p. inoculated into $CN^{+/+}$ mouse, a number of microvilli were induced on the surface of peritoneal MS cells, while many microvilli were always prominently present in $CN^{-/-}$ mice even without the inoculation of F10 cells (data not shown). It has been reported that the appearance of microvilli is associated with reduced intercellular contact and destabilization of the actin cytoskeleton.[32,33] We therefore assume that the microvilli observed in $CN^{-/-}$ mice reflected the weakened intercellular contact between MS cells. Thus, the actin filaments stabilized by calponin h1 gene transfer in the present study may be capable of strengthening intercellular adhesion of MS cells and protecting the peritoneum from the invasion of F10 cells.

Rho-activated cells that produce a marked development of actin filaments and the cell contraction through the actomyosin system are reported to interfere with adherence;[34] consequently those cells are metastatic in nature.[35–37] Contrarily, the calponin homology (CH) domain in *vav* is reported to suppress the Rho family signaling pathway.[38,39] These reports led us to speculate that actin fibers developed in Rho-activated cells are capable of promoting dynamic cellular activity, which is different from that induced by calponin h1, which is static in order to suppress cellular activity. In fact, exogenous expression of calponin h1 has been found to reduce the motility of human fibrosarcoma[8] and src-transformed 3Y1 cells,[40] and the expression of calponin h1 in NIH3T3 fibroblasts reduced cell motility in wound healing assays.[29]

Our study demonstrated that calponin h1 functions to suppress the proliferation of F10 cells (data not shown) by gene transfer experiments, consistent with other studies.[7,8,41,42] Furthermore, we recently found that calponin h1 inhibited the expression of vascular endothelial growth factor and consequently suppressed the angiogenic activity in src-transformed fibroblasts.[40] The anti-angiogenic factor is reported to protect against the peritoneal dissemination of cancer cells.[43] Thus, calponin h1 exerts a bifunctional action, that is, to protect the host against cancer invasion by recovering the MS cells itself and intercellular fragility of MS cells and also to inhibit the growth of cancer cells.

Combining the results of our study with previous studies, we conclude that calponin h1 strengthens the function of the peritoneum as a barrier against the invasion of cancer cells, besides inhibiting the proliferation of cancer cells. Bifunctional genes such as calponin h1 may herald the arrival of new type of gene therapy that can both protect the host and attack cancer cells.

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