Role of vimentin in smooth muscle force development

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Wang, Ruping, Qingfen Li, and Dale D. Tang. Role of vimentin in smooth muscle force development. Am J Physiol Cell Physiol 291: C483–C489, 2006.—Vimentin intermediate filaments undergo spatial reorganization in cultured smooth muscle cells in response to contractile activation; however, the role of vimentin in the physiological properties of smooth muscle has not been well elucidated. Tracheal smooth muscle strips were loaded with antisense oligonucleotides (ODNs) against vimentin and then cultured for 2 days to allow for protein degradation. Treatment with vimentin antisense, but not sense, ODNs suppressed vimentin protein expression; neither vimentin antisense nor sense ODNs affected protein levels of desmin and actin. Force development in response to ACh stimulation or KCl depolarization was lower in vimentin-deficient tissues than in vimentin sense ODN- or non-ODN-treated muscle strips. Passive tension was also depressed in vimentin-depleted muscle tissues. Vimentin downregulation did not attenuate increases in myosin light chain (MLC) phosphorylation in response to contractile stimulation or basal MLC phosphorylation. In vimentin sense ODN-treated or non-ODN-treated smooth muscle strips, the desmosomal protein plakoglobin was primarily localized in the cell periphery. The membrane-associated localization of plakoglobin was reduced in vimentin-depleted muscle tissues. These studies suggest that vimentin filaments play an important role in mediating active force development and passive tension, which are not regulated by MLC phosphorylation. Vimentin downregulation impairs the structural organization of desmosomes, which may be associated with the decrease in force development.

intermediate filaments; cytoskeleton; contraction; desmin

THE INTERMEDIATE FILAMENT network is one of three cytoskeletal systems in smooth muscle cells. Intermediate filaments link to the membrane at the desmosome, an intercellular junction. At the desmosome, the cytoplasmic domains of transmembrane desmoscin and desmoglein connect with intermediate filaments via desmosomal proteins such as plakoglobin. The extracellular domains of desmocollin and desmoglein engage with their counterparts in adjacent cells to form the intercellular connection. In addition, in the myoplasm, intermediate filaments connect to dense bodies to which actin filaments also attach (1, 8, 15, 25).

Five types of structurally related intermediate filament proteins have been identified; their expression is cell-type specific (13). Vimentin is a major intermediate filament protein in airway and vascular smooth muscle, whereas desmin is expressed in airway smooth muscle and microarteries (12, 14, 28, 39).

We previously showed that contractile stimulation leads to vimentin phosphorylation at Ser56 and spatial reorientation of the vimentin network in cultured smooth muscle cells (28). In addition, ACh stimulation of canine tracheal smooth muscle strips increases the amount of soluble vimentin (33), indicating that vimentin may undergo remodeling in differentiated smooth muscle tissues. Spatial reorganization of vimentin intermediate filaments also occurs in endothelial cells and fibroblasts in response to stimulation with platelet-derived growth factor and epidermal growth factor. Remodeling of vimentin filaments has been implicated in regulation of endothelial cell and fibroblast division (19, 38).

Plakoglobin plays a pivotal role in mediating desmosomal formation in certain cell types (3, 6). In human fibrosarcoma cells and COS-7 cells, overexpression of plakoglobin promotes clustering of desmosomal plaque complexes at cell-cell borders (3). Biochemical and morphological analysis indicates that junctional incorporation of the desmosomal component is impaired in plakoglobin-deficient murine keratinocytes. Reexpression of plakoglobin in these plakoglobin-deficient cells largely reverses these effects (40).

In this study, the role of vimentin in smooth muscle was assessed by evaluation of the effects of antisense oligonucleotide (ODN)-induced vimentin downregulation on the physiological properties of smooth muscle. Our results suggest that vimentin is involved in passive tension and active force development and membrane-associated plakoglobin distribution, but not myosin light chain (MLC) phosphorylation, in smooth muscle, indicating that vimentin intermediate filaments are involved in force development in smooth muscle tissues.

MATERIALS AND METHODS

Preparation of smooth muscle tissues. Mongrel dogs (20–25 kg body wt) were anesthetized with pentobarbital sodium (30 mg/kg iv) and quickly exsanguinated. All experimental protocols were approved by the Institutional Animal Care and Use Committee. A 15-cm segment of extrathoracic trachea was immediately removed and placed at room temperature in physiological saline solution (PSS; in mM: 110 NaCl, 3.4 KCl, 2.4 CaCl2, 0.8 MgSO4, 25.8 NaHCO3, 1.2 KH2PO4, and 5.6 glucose). The solution was aerated with 95% O2-5% CO2 to maintain pH at 7.4. Rectangular 0.6- to 0.8-mm-wide, 0.2- to 0.3-mm-thick, and 9- to 10-mm long tracheal smooth muscle strips were dissected from the trachea after removal of the epithelium and connective tissue layer. Use of canine tracheal muscle strips of the aforementioned width, thickness, and length was critical for maintenance of muscle contractility during the incubation period and for successful introduction of ODNs throughout the muscle strip.

Measurement of tension development in smooth muscle. Each muscle strip was placed in PSS at 37°C in a 25-ml organ bath and attached to a force transducer (Grass) that had been connected to a recorder (Gould) or a computer with an analog-to-digital converter (Grass). At the beginning of each experiment, the muscle strips were stretched to the reference muscle length (9–10 mm). After 30 min of equilibration, the muscle strips were stimulated repeatedly with 10–5 mol/L ACh.

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M ACh until contractile responses and passive tension reached steady state. In untreated muscle tissues, average passive tension was 0.2 g, and average active force was 4 g. ODNs dissolved in Tris-EDTA buffer were introduced into the muscle strips according to experimental procedures described previously (29, 36). Muscle strips were then incubated for 2 days with ODNs in Dulbecco's modified Eagle's medium. The muscle strips were returned to PSS at 37°C in 25-ml organ baths and stretched to the corresponding reference muscle length. After repeated stimulation with ACh, each contractile response and each passive tension was compared with the corresponding preincubation value. For biochemical analysis, muscle strips were frozen using liquid N₂-cooled tongs and then pulverized under liquid N₂ using a mortar and pestle.

Loading of ODNs and organ culture. Antisense ODNs with the following sequence were designed to selectively suppress vimentin expression in canine tracheal smooth muscle: 5′-AGGACCCGAGC-CTGGTG-G-3′ (based on the human vimentin cDNA sequence; GenBank accession no. XM 167414). The vimentin sense sequence (5′-CCACCCAGTGCTGTCCT-3′) and the scrambled sequence (5′-CGAAATCCTGAGGGTA-3′) were used as control. According to sequence-matching results from The National Center for Biotechnology Information, these sequences are not homologous to those of any other contractile or cytoskeletal proteins. The antisense molecule targets to a region of mRNA that is unique to vimentin. The phosphorothioate ODNs were synthesized and purified by Invitrogen (Carlsbad, CA). The ODNs were introduced into the smooth muscle strips by chemical loading (also referred to as reversible permeabilization) using methods we have previously described (29, 34, 36).

Analysis of protein expression. Pulverized muscle strips were mixed with 50 μl of extraction buffer containing 20 mM Tris-HCl (pH 7.4), 2% Triton X-100, 0.2% SDS, 2 mM EDTA, phosphatase inhibitors (2 mM sodium orthovanadate, 2 mM molybdate, and 2 mM sodium pyrophosphate), and protease inhibitors (2 mM benzamidine, 0.5 mM aprotinin, and 1 mM phenylmethylsulfonyl fluoride). Each sample was kept on ice for 1 h and then centrifuged for the collection of supernatant. Muscle extracts were boiled in sample buffer [1.5% DTT, 2% SDS, 80 mM Tris-HCl (pH 6.8), 10% glycerol, and 0.01% bromphenol blue] for 5 min and then separated by SDS-PAGE. Proteins were transferred to a nitrocellulose membrane, which was cut into two parts for immunoblotting of different proteins. The upper part of the membrane was blocked with 5% milk for 1 h and probed with monoclonal antibody against vimentin (BD Pharmingen, San Diego, CA) followed by horseradish peroxidase-conjugated anti-mouse IgG (Amersham Pharmacia Biotech, Piscataway, NJ). The nitrocellulose membranes were then stripped of bound antibodies and reprobed with desmin monoclonal antibody (BD Pharmingen). The lower part of the membrane was reacted with a monoclonal antibody against actin (clone 1A4, Sigma) and then with horseradish peroxidase-conjugated anti-mouse IgG. Proteins were visualized by enhanced chemiluminescence and quantitated by scanning densitometry. Densitometric values of vimentin, desmin, and actin were determined for sense- and antisense-treated strips and normalized to those of non-ODN-treated strips. The ratios of these proteins were calculated to verify that changes in protein expression were selective for vimentin.

Electron microscopy of smooth muscle tissues. Tracheal smooth muscle strips were placed in organ baths containing PSS and fixed at the reference length at 37°C for 15 min in PSS containing 2% glutaraldehyde, 2% paraformaldehyde, and 2% tannic acid in 0.1 M sodium cacodylate buffer (pH 7.2). The samples were removed from the organ baths, cut into small blocks (1 × 0.5 × 0.1 mm), placed in the same fixing solution for 2 h at 4°C, and washed three times for 10 min each in 0.1 M sodium cacodylate buffer. The tissues were then fixed with 1% OsO₄ in 0.1 M sodium cacodylate buffer for 2 h. After the samples were washed three times with distilled water, they were stained en bloc in 1% uranyl acetate for 1 h, washed and dehydrated through a graded series of ethanol (50, 70, 80, 90, and 95%) for 10 min each and then with 100% ethanol, and embedded in Spurr's resin. Ultrathin (~90-nm) sections were cut with a diamond knife and picked up on Formvar-coated grids. The sections were stained in 1% uranyl acetate followed by lead citrate and examined on a transmission electron microscope (model 100CX, JEOL).

Assessment of MLC phosphorylation. MLC phosphorylation in tracheal smooth muscle strips was assessed by immunoblot analysis as described previously (29–32, 37).

Cell dissociation and immunofluorescence analysis. Tracheal smooth muscle strips were minced and transferred to 5 ml of dissociation solution containing 130 mM NaCl, 5 mM KCl, 1.0 mM CaCl₂, 1.0 mM MgCl₂, 10 mM HEPES, 0.25 mM EDTA, 10 mM d-glucose, 10 mM taurine (pH 7), collagenase (400 U/ml, type 1), papain (30 U/ml, type IV), BSA (1 mg/ml), and DTT (1 mM). All enzymes were obtained from Sigma. The strips were placed in a 37°C shaking water bath at 80 oscillations per minute for 10 min, washed three times with a HEPES-buffered saline solution [in mM: 130 NaCl, 5 KCl, 1.0 CaCl₂, 1.0 MgCl₂, 20 HEPES, and 10 d-glucose (pH 7.4)], and triturated with a pipette to liberate individual smooth muscle cells from the tissue. The solution containing the dissociated cells was poured over slides, and the cells were allowed to adhere to the slides for 2–3 h at room temperature (20).

The smooth muscle cells were fixed for 10 min in 4% paraformaldehyde, washed three times in Tris-buffered saline (50 mM Tris, 150 mM NaCl, and 0.1% NaN₃), and permeabilized with 0.2% Triton X-100 dissolved in TBS for 5 min. The cells were then incubated with vimentin monoclonal antibody (BD Pharmingen; 1:100 dilution) or desmin goat polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA; 1:20 dilution) for 45 min at 37°C. The cells were washed and incubated with a secondary antibody conjugated to Alexa 488 or Alexa 546 fluoroprobe (Molecular Probes, Eugene, OR) for 30 min at 37°C. The cells were washed again, and the slides were covered with coverslips using Fluoromount-G. The cellular localization of fluorescently labeled proteins was viewed under laser scanning confocal microscopy (model LSM 510, Zeiss) using an Apo ×63 oil immersion objective. Alexa 488-labeled proteins (green) were excited with a 488-nm argon laser light, and fluorescent emissions were collected at 500–550 nm. The fluorescence of Alexa 546-labeled protein (red) was excited with a helium-neon laser at 543 nm, and emissions were collected at 565–615 nm.

Immunohistochemistry. Tracheal smooth muscle tissues were embedded in frozen tissue-embedding medium (Neg 52, Richard-Allen Scientific) and sectioned using cryostats (Richard-Allen Scientific). The sections were air-dried and incubated with plakoglobin monoclonal antibody (BD Pharmingen; 1:20 dilution) and vinculin rabbit polyclonal antibody (Santa Cruz Biotechnology; 1:100 dilution) at room temperature for 1 h. The sections were then washed with PBS and incubated with a secondary antibody conjugated to Alexa 488 or Alexa 546 fluoroprobe for 30 min at 37°C. The samples were covered and viewed under laser scanning confocal microscopy.

Image analysis for protein distribution was performed using the previously described method with modification (20). Briefly, images of smooth muscle cells in the tissues were analyzed for distribution differences of stained proteins by quantification of the pixel intensity with a series of three to four line scans across the boarder of cells in tissue cross sections. The ratio of pixel intensity at the cell border to pixel intensity in the cell interior was determined for each line scan as follows: ratio of the average maximal pixel intensity at the cell periphery to minimal pixel intensity in the cell interior. The ratios of pixel intensity at the cell periphery to that in the cell interior for all the line scans performed on a given cell were averaged to obtain a single value for the ratio of each cell.

Statistical analysis. All statistical analysis was performed using Prism 4 software (GraphPad Software, San Diego, CA). Comparison among multiple groups was performed by one-way analysis of variance followed by a post test (Tukey’s multiple comparison test).
Differences between pairs of groups were analyzed by Student-Newman-Keuls test or Dunn’s method. Values of \( n \) refer to the number of experiments used to obtain each value. \( P < 0.05 \) was considered to be significant.

RESULTS

Spatial distribution of vimentin and desmin in freshly dissociated smooth muscle cells. We evaluated the spatial distribution of the type III intermediate filament proteins vimentin and desmin in freshly dissociated tracheal smooth muscle cells. Smooth muscle cells were freshly dissociated from canine tracheal smooth muscle cells and immunostained for vimentin and desmin. Protein distribution in these cells was viewed under a confocal fluorescence microscope. Vimentin was mainly localized in the myoplasm, whereas desmin was primarily detected on the cell periphery. Vimentin and desmin were not found in the nucleus of the freshly dissociated smooth muscle cells (Fig. 1).

Vimentin downregulation attenuates force development in response to stimulation with ACh. To evaluate the role of vimentin in smooth muscle, vimentin antisense or sense ODNs or scrambled ODNs were introduced into canine tracheal smooth muscle strips by chemical loading (29, 31, 32, 36). These muscle strips were incubated for 2 days to allow for protein depletion. Protein expression from smooth muscle tissues was assessed by immunoblot analysis. The expression of vimentin protein was lower in muscle tissues treated with antisense ODNs than in tissues treated with sense or scrambled ODNs or in non-ODN-treated tissues. However, the expression of desmin and \( \alpha \)-actin was not affected by antisense treatment (Fig. 2, A and B; \( n = 4 – 6 \)).

We also assessed the effects of vimentin antisense ODNs on the ultrastructure of smooth muscle tissues. Electron-microscopic analysis revealed that the amount of intermediate fila-
ments was reduced in antisense-treated tissues compared with control tissues. However, the organization of thin and thick filaments in smooth muscle tissues treated with vimentin antisense was not markedly different from that in tissues treated with sense ODNs (Fig. 2, C and D).

We evaluated isometric force development in response to ACh in non-ODN-treated and vimentin sense- or antisense ODN-treated smooth muscle strips. Active stress of the tracheal muscle strips in response to 5 min of stimulation with 10^{-5} M ACh is 95–100 mN/mm² before incubation (20, 29, 30, 33, 34, 36, 42). Force development in response to 10^{-5} M ACh was compared before and after 2 days of incubation. In non-ODN-treated muscle strips, contractile force in response to 5 min of ACh stimulation was similar to preincubation force (n = 10, P > 0.05). Contractile response in vimentin sense-treated tissues was 97% of preincubation force (n = 10, P > 0.05). However, ACh-stimulated force in antisense-treated tissues was significantly reduced to 24% of the corresponding preincubation force (n = 10, P < 0.01; Fig. 3).

Antisense inhibition of vimentin depresses passive tension in smooth muscle. We assessed passive tension in vimentin antisense- or sense ODN-treated and non-ODN-treated smooth muscle strips. Without ODN treatment, passive tension was similar before and after incubation (n = 10, P > 0.05). The passive strain in vimentin sense-treated tissues was also not distinguishable before and after incubation (n = 10, P > 0.05). In contrast, passive tension in antisense-treated tissues was reduced after the incubation period compared with preincubation strain (n = 10, P < 0.01; Fig. 4).

MLC phosphorylation is not attenuated in vimentin-deficient smooth muscle. MLC phosphorylation has been thought to mediate tension development in smooth muscle (26, 29). To determine whether vimentin depletion affects MLC phosphorylation, a subset of vimentin antisense- or sense ODN-treated and non-ODN-treated tracheal smooth muscle strips was stimulated with 10^{-5} M ACh for 5 min or left unstimulated and then frozen for analysis of MLC phosphorylation.

Although passive tension was significantly depressed, basal MLC phosphorylation in antisense-treated tissues was not significantly distinguishable from that in non-ODN-treated or sense ODN-treated muscle strips (Fig. 5; n = 4, P > 0.05). In addition, active force was suppressed in vimentin-deficient tissues. However, ACh stimulation significantly increased MLC phosphorylation in tissues treated with vimentin antisense ODNs. The average increases in MLC phosphorylation in non-ODN-treated or sense- and antisense-treated tissues were not significantly different 5 min after ACh stimulation (Fig. 5; n = 4, P > 0.05).

Vimentin depletion inhibits contractile force without attenuating increases in KCl-stimulated MLC phosphorylation. We also assessed the effect of vimentin depletion on force development and MLC phosphorylation in KCl-stimulated tracheal smooth muscle strips. Force and MLC phosphorylation were measured 5 min after stimulation (22, 29, 36). Contractile force
in response to KCl stimulation was lower in antisense-treated than in non-ODN-treated or sense ODN-treated muscle strips (Fig. 6A). Increases in MLC phosphorylation in non-ODN-treated muscle tissues were similar to those in vimentin sense- or antisense ODN-treated muscle strips (Fig. 6B; \( P > 0.05 \)).

**Downregulation of vimentin disrupts membrane-associated distribution of plakoglobin in smooth muscle tissues.** We assessed the subcellular localization of plakoglobin in smooth muscle tissues exposed to vimentin antisense or sense ODNs. Vimentin antisense- or sense ODN-treated or non-ODN-treated smooth muscle strips were cross sectioned and immunofluorescently labeled for plakoglobin. As a control, these sections were also immunostained for vinculin, a cytoskeletal protein. Subcellular localization of these proteins was viewed under a laser scanning fluorescence microscope and quantitatively analyzed (see MATERIALS AND METHODS).

Plakoglobin staining in cells from non-ODN-treated tissues was detected primarily at the cell periphery (Fig. 7Aa). In vimentin sense-treated tissues, plakoglobin was also localized on the membrane (Fig. 7Ab). However, the membrane distribution of plakoglobin in muscles treated with vimentin antisense ODNs was reduced dramatically (Fig. 7Ac). The subcellular localization of vinculin was comparable in non-ODN-treated and vimentin sense- or antisense ODN-treated tissues (Fig. 7A, a', b', c').

A total of 20 non-ODN-treated cells, 20 sense-treated cells, and 20 antisense-treated cells from 3 experiments were quantitatively analyzed for protein distribution. The ratio of plakoglobin fluorescence intensity at the cell periphery to that in the interior in tissues treated with vimentin sense ODNs was not significantly different from that in non-ODN-treated tissues (\( P > 0.05, n = 20 \)). However, the fluorescence intensity ratio was significantly lower in vimentin antisense-treated than in non-ODN- or vimentin sense ODN-treated tissues (\( P < 0.05, n = 20 \)). Pixel ratios of cell border to interior for the focal adhesion-associated protein vinculin were not statistically different among non-ODN-treated or vimentin sense- or antisense ODN-treated smooth muscle tissues (\( P > 0.05, n = 20 \)).

**DISCUSSION**

Our present results demonstrate that vimentin downregulation in smooth muscle affects passive tension, active force, and plakoglobin distribution, but not MLC phosphorylation. We propose that vimentin may play an essential role in tension development in smooth muscle that is not mediated by MLC phosphorylation.

Although it has long been shown that vimentin and desmin are major intermediate filament proteins in smooth muscle, their spatial distribution in differentiated smooth muscle cells is not fully understood. In this report, we have observed that vimentin is mainly localized in the myoplasm, whereas desmin is primarily distributed in the cell cortex. These results lead us to suggest that vimentin filaments in smooth muscle cells may link to dense bodies and to desmosomes, whereas desmin filaments localized in the cell periphery may reinforce the linkage of vimentin filaments to the desmosomes.

The primary function of the intermediate filament network has been thought to be maintenance of cell shape and tissue integrity (1, 8, 25). To evaluate the role of the intermediate filament protein vimentin, smooth muscle strips were treated with antisense ODNs against vimentin. Treatment with antisense ODNs selectively downregulated vimentin protein expression. Moreover, electron-microscopic analysis revealed that the ultrastructural organization of contractile filaments was not disrupted in smooth muscle tissues treated with vimentin antisense ODNs. Microfilaments of astrocytes from vimentin-knockout mice have also been shown to be similar to those from wild-type mice (16). Vimentin downregulation inhibited passive tension and active force in response to stimulation with the muscarinic agonist or KCl depolarization. These results suggest that depression of basal tension and active force cannot be attributed to a general decline in protein expression or disruption of contractile filaments in smooth muscle tissues treated with vimentin antisense ODNs.

Phosphorylation of the 20-kDa regulatory light chain of myosin by Ca\(^{2+}\)/calmodulin-regulated MLC kinase initiates cross-bridge cycling and smooth muscle contraction (26, 27). The state of MLC phosphorylation can also be regulated by Rho kinase (26). Furthermore, the subcellular localization of vimentin filaments affects the distribution of Rho kinase in HeLa cells (24). Thus we considered the possibility that depression of active force development in vimentin-deficient tissues in response to activation with contractile stimuli could be due to inhibition of contractile protein activation. However, MLC phosphorylation in response to contractile stimulation is normal, whereas active force in the vimentin-depleted strips is dramatically depressed. Additionally, basal MLC phosphorylation has been thought to be associated with passive tension. In this report, passive tension, but not basal MLC phosphory-

**Fig. 6. Contractile force and MLC phosphorylation in vimentin-deficient KCl-stimulated muscle strips. A:** smooth muscle strips were contracted with 60 mM KCl before and after 2 days of incubation without ODNs or with vimentin sense or antisense, and mean active force in response to 60 mM KCl was quantified as percentage of KCl-induced force in each strip before incubation. Values are means \( \pm SE \) (\( n = 6–7 \)). *Significantly different from No ODNs and Vim S (\( P < 0.05 \)). **B:** muscle strips were incubated without ODNs or with vimentin sense or antisense, and MLC phosphorylation was measured 5 min after stimulation with 60 mM KCl. Values are means \( \pm SE \) (\( n = 4 \)). Differences between antisense-, sense-, and non-ODN-treated strips were not statistically significant.
Desmosomes are complex intercellular junctions specialized to provide strong but dynamic cell-cell adhesion in a variety of cell types and tissues, including smooth muscle cells (15, 18, 25). As a major component of the desmosomes, plakoglobin links intermediate filaments to desmosomal cadherins (desmoglein and desmocollin) on the membrane and has been used as a desmosomal marker (15, 25). Lack of plakoglobin in murine keratinocytes impairs the junctional incorporation of desmoglein and desmocollin (40). Overexpression of plakoglobin in human fibrosarcoma and COS-7 cells promotes clustering of desmosomal plaque at intercellular borders (3). In this report, downregulation of vimentin in smooth muscle impaired the membrane-associated localization of plakoglobin, indicating that the structure of desmosomes may be disrupted in vimentin-deficient smooth muscle cells. The disruption of desmosomal assembly may affect intercellular mechanical transduction and, thus, force development in these smooth muscle tissues.

Contractile stimulation induces the spatial reorganization of vimentin intermediate filaments in cultured smooth muscle cells. The spatial rearrangement of vimentin filaments may be the part of cytoskeletal remodeling that affects force development during activation with contractile agonists (28). In addition, Ca²⁺/calmodulin-dependent kinase IIγ (CamKIIγ) has been shown to regulate arterial smooth muscle contraction (23). CamKIIγ G-2 (a novel form of CamKII) is associated with vimentin filaments in unstimulated differentiated arterial smooth muscle cells. On contractile stimulation, CamKIIγ G-2 is released into the cytosol and, subsequently, targeted to cortical dense plaques, which has been proposed to be an important signaling process in smooth muscle cells (17). Thus it is possible that the structural reorganization of the vimentin network may facilitate the redistribution of signaling molecules such as CamKIIγ G-2 during contractile activation of smooth muscle. Deficiency of vimentin protein may impair the translocation of certain signaling molecules in smooth muscle in response to contractile activation.

The intermediate network has been proposed to interact with the actin cytoskeleton in certain cell types, including macrophages, epithelial cells, and fibroblasts (7, 10, 11). Treatment of epithelial cells with cytochalasin D, which is known to inhibit actin polymerization, disrupts the organization of the keratin intermediate filament network (10). Recent studies have also shown that contractile activation of smooth muscle induces actin polymerization, which may be associated with tension development in smooth muscle (2, 9, 20, 30, 34, 37, 42). Although disruption of actin stress fibers does not affect the reorganization of vimentin filaments in cultured smooth muscle cells and HeLa cells (4, 28), we do not rule out the possibility that lack of vimentin protein may interfere with reorganization of the actin cytoskeleton in vimentin-deficient smooth muscle strips in response to contractile stimulation.

In fibroblasts, intermediate filaments and microtubules form closely associated parallel arrays that are distributed throughout the cytoplasm. Treatment with the microtubule disruption agents nocodazole or colchicines induces vimentin filament reorganization (5). Thus we speculate that vimentin deficiency could reciprocally influence the structural array of microtubules in these smooth muscle strips, which might be associated with the decrease in force development. However, reports on the role of microtubules in smooth muscle contraction are controversial (21, 41). Treatment with nocodazole slightly enhanced KCl-induced vascular muscle contraction by increasing intracellular Ca²⁺, not by affecting mechanical stiffness.

In summary, the downregulation of vimentin in smooth muscle inhibits passive tension and active force development, but not MLC phosphorylation. The lack of vimentin protein decreases the membrane-associated distribution of plakoglobin, a desmosomal protein. We conclude that vimentin is necessary for force development in smooth muscle, which is not regulated by the state of MLC phosphorylation. Vimentin deficiency impairs the structural organization of desmosomes, which may be associated with the decrease in force development.
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