Reorganization of the Vimentin Network in Smooth Muscle

Vimentin intermediate filaments (IFs) link to desmosomes (intercellular junctions) on the membrane and dense bodies in the cytoplasm, which provides a structural base for intercellular and intracellular force transmission in smooth muscle. There is evidence to suggest that the vimentin framework plays an important role in mediating smooth muscle mechanical properties such as tension and contractile responses. Contractile activation induces vimentin phosphorylation at Ser-56 and vimentin network reorientation, facilitating contractile force transmission among and within smooth muscle cells. P21-activated kinase 1 and polo-like kinase 1 catalyze vimentin phosphorylation at Ser-56, whereas type 1 protein phosphatase dephosphorylates vimentin at this residue. Vimentin filaments are also involved in other cell functions including migration and nuclear positioning. This review recapitulates our current knowledge how the vimentin network modulates mechanical and biological properties of smooth muscle. [DOI: 10.1115/1.4042313]

Introduction

The type III intermediate filament (IF) proteins vimentin and desmin are present in airway smooth muscle cells/tissues. However, vimentin is a predominant IF protein in airway smooth muscle; the protein ratio of vimentin to desmin is 6:1 [1]. Vimentin exists in the form of a filamentous structure emanating from the nucleus and connecting to the desmosome on the membrane. At the desmosome, vimentin filaments interact with the linker proteins such as plakoglobin, plakophilin, and desmolplakin, which in turn connect with the cytoplasmic tails of desmosomal cadherins (desmocollin and desmoglein). The extracellular domains of desmocollin and desmoglein connect with their counterparts in adjacent cells to form the cell–cell junction (Fig. 1) [2]. Additionally, another end of vimentin filaments attaches to the cytoplasmic dense bodies to which actin filaments also connect. Thus, the vimentin network may facilitate intercellular and intracellular mechanical transmission. Furthermore, desmin is primarily localized in the peripheral of airway smooth muscle cells, which may strengthen the connection of vimentin filaments to the membrane [1,3,4]. In contrast, actin filaments of smooth muscle link to membrane-associated dense plaques, which include integrins and linker proteins, and engage with the extracellular matrix (ECM) (Fig. 1). A detailed structure of membrane-associated dense plaques (also called the integrin-associated complex) has been described elsewhere [5]. The membrane-associated dense plaques play a role in force transmission between the contractile units and the ECM [5–9].

Over the last decade, much work has been done to understand mechanical and physiological properties of vimentin intermediate filaments in smooth muscle and other cell types. Because the theme of this special issue is airway smooth muscle and airway mechanics, we will recapitulate the role of the vimentin network in the airway smooth muscle mechanical property and signaling pathways that regulate vimentin functions. Additionally, we will summarize our current understanding of how the vimentin network may affect other biological properties of smooth muscle.

Role and Regulation of the Vimentin Network in the Mechanical Properties of Smooth Muscle

Vimentin Filaments and Smooth Muscle Mechanical Properties. Smooth muscle tension and contraction play an essential role in regulating functions of pulmonary and cardiovascular systems such as airway tone and blood pressure. There is an accumulating evidence to suggest an important role for vimentin filaments in controlling mechanical properties of smooth muscle. Vimentin knockout does not affect mouse viability and...
reproductivity; however, flow-induced dilation in mesenteric resistance arteries was reduced in vimentin knockout mice [10]. Moreover, vimentin depletion by antisense oligonucleotides inhibits smooth muscle contraction during agonist activation [4]. Vimentin-mediated contraction is not orchestrated by contractile protein activation because myosin light chain phosphorylation is not affected by vimentin depletion [1,14]. The structure of desmosomes is also disrupted in vimentin-deficient tissues as evidenced by the immunostaining of plakoglobin, a major component of desmosomes [1,3,4]. Thus, vimentin filaments facilitate smooth muscle contraction by stabilizing intercellular and intracellular mechanical transmission [1–3,11]. The vimentin network also affects the mechanical stability of nonmuscle cells. Fibroblasts from vimentin −/− mice display reduced stiffness, mechanical stability, and contraction [12]. Disruption of the vimentin network by withaferin-A reduces stiffness of NK cells [13]. However, the information regarding the role of the vimentin network in other airway smooth muscle biophysical properties (e.g., stiffness and traction force) is lacking.

The vimentin network of nonmotel cells has long been thought to be a fixed structure [1,14]; however, investigations in the last decade demonstrate that vimentin intermediate filaments are dynamic in various cell types including smooth muscle cells upon external stimulation. Contractile activation induces vimentin phosphorylation at Ser-56 and reorganization of the vimentin framework in smooth muscle [1,3,15,16]. Vimentin phosphorylation at this residue influences the reorganization of the vimentin network. Introduction of a nonphosphorylatable S56A vimentin inhibits vimentin cytoskeletal remodeling and smooth muscle contraction [1,3,4,15]. Because vimentin filaments connect with the desmosome and dense bodies, reorganization of the vimentin network may strengthen the intercellular mechanical transmission and optimize arrangement of contractile elements, which may promote force development [1,11]. In addition, vimentin phosphorylation at Ser-56 regulates the endothelial barrier function, which is associated with cell contraction. Disruption of vimentin filaments by withaferin A or introduction of the nonphosphorylatable vimentin mutant S56A impairs the barrier function of endothelial cells [17].

The vimentin cytoskeleton also harbors certain signaling partners such as a p130 Crk-associated substrate (p130CAS) in smooth muscle [3,15,18]. p130CAS is an adapter protein that promotes actin dynamics and smooth muscle contraction by activating N-WASP [19,20] and profilin-1 [21]. Phosphorylation of vimentin at Ser-56 leads to the dissociation of p130CAS from the vimentin cytoskeleton during contractile activation [3,15]. Expression of S56A vimentin inhibits the dissociation of p130CAS from cytoskeletal vimentin [15]. Moreover, p21-activated kinase 1 (PAK1) can mediate vimentin phosphorylation at Ser-56 in smooth muscle [3,22] (see below). PAK1 depletion by antisense oligodeoxynucleotides attenuates the agonist-induced release of p130CAS from the vimentin cytoskeleton [1,13]. Thus, it has been proposed that the vimentin cytoskeleton regulates actin cytoskeleton reorganization by modulating vimentin phosphorylation-dependent p130CAS release; the released p130CAS translocates to the plasma membrane to facilitate cortical actin polymerization, which promotes the transmission of contractile force to the ECM as well as cadherin-mediated intercellular mechanical transmission and smooth muscle contraction [1,3,5,15,23].

Although actin filaments indirectly interact with the vimentin network (Fig. 1), the actin cytoskeleton does not affect the vimentin framework. Treatment with the actin polymerization inhibitor cytochalasin D does not affect the integrity of the vimentin framework [1,16]. In addition, actin cytoskeletal reorganization is largely regulated by protein tyrosine phosphorylation [5–7]. Treatment with a protein tyrosine kinase inhibitor attenuated phosphorylation of Paxillin and p130CAS (two important tyrosine-phosphorylated protein for actin cytoskeleton reorganization); however, the tyrosine kinase inhibitor did not affect reorganization of vimentin intermediate filaments [1,16,24].

The vimentin network and microtubules form parallel arrays that are distributed throughout the cytoplasm of nonmuscle cells such as fibroblasts and epithelial cells [25,26]. Disruption of microtubules by the inhibitors nocodazole, colchicine, or colcemid induced vimentin filament rearrangement in nonmuscle cells [25]. If microtubules affect the reorganization of vimentin intermediate filaments, treatment to nocodazole should attenuate vimentin cytoskeleton remodeling and contraction. However, in two separate studies, exposure to nocodazole slightly enhanced KCl-induced vascular smooth muscle contraction, which may be due to increase in intracellular Ca2+ [27,28]. These studies suggest that microtubule-dependent regulation of the vimentin network may be cell-type specific.

The vimentin framework also interacts with calcium/calmodulin-dependent protein kinase II (CamKII) in vascular smooth muscle cells [29]. CamKII promotes smooth muscle contraction by activating mitogen-activated protein kinase (MAPK), which in turn phosphorylates caldesmon [1,29]. Phosphorylated caldesmon promotes myosin ATPase or actin filament remodeling and smooth muscle contraction [29,30]. Contractile activation induces the redistribution of CamKII from vimentin-containing cytoskeletal scaffold to the cortical region, which may activate ERK1/2 activation and contraction [1,29].

Regulation of Vimentin Filaments in Smooth Muscle. p21-activated kinase (PAK) plays an important role in regulating vimentin phosphorylation at Ser-56 in smooth muscle during contractile activation. Although six isoforms of PAK have been found in mammalian cells, PAK1 is a predominant isoform in smooth muscle [1,3,15,31]. Contractile activation of smooth muscle induces PAK1 phosphorylation at Thr-423, an indication of PAK1 activation. Knockdown of PAK1 inhibits the agonist-induced vimentin phosphorylation, partial disassembly of vimentin filaments, and dissociation of p130CAS from cytoskeletal vimentin, which may result in a reduction of contractile force [1,3,15,16].

The upstream molecules for activating PAK1 in smooth muscle have been also identified. They are Cdc42 (one of small GTPases), Cdc42GAP (GTPase activating protein), reactive oxygen species (ROS), and NAD(P)H oxidase. The small GTPase can bind to the p21-binding domain of PAK, which induces conformational changes and activates the kinase through autophosphorylation at Thr-423 [1,3,15,31]. Cdc42 is regulated by Cdc42GAP. The addition of ROS inhibits the activation of Cdc42GAP in smooth muscle. There is also evidence to suggest that ROS regulates the activity of Cdc42GAP. The addition of ROS inhibits the activation of Cdc42GAP in smooth muscle cells. Moreover, knockdown of p47phox (a regulatory subunit of NAD(P)H oxidase) by shRNA blocks ROS production and inhibits the GAP protein. The disinhibition of Cdc42GAP can be rescued by the addition of hydrogen peroxide. Cdc42 activity, PAK1 phosphorylation, vimentin phosphorylation, and smooth muscle contraction were reduced by p47phox knockdown [5,6,22,32]. Together, these studies discover a novel pathway for the regulation of the vimentin network. Contractile activation increases the activity of NAD(P)H oxidase, which promotes the production of intracellular ROS. ROS can inhibit the activity of Cdc42GAP, which subsequently induces Cdc42, PAK1, and vimentin phosphorylation and reorganization [5,6,22,32] (Fig. 2).

Polo-like kinase 1 (Plk1) is a serine/threonine protein kinase that has been implicated in mitosis and cytokinesis [33,34]. Recent studies suggest that Plk1 also orchestrates the phosphorylation of vimentin at Ser-56. Knockdown of Plk1 by lentivirus-mediated shRNA inhibits vimentin phosphorylation and the contraction of human airway smooth muscle upon agonist stimulation [11]. Contractile stimulation enhances the phosphorylation and activation of Plk1 in airway smooth muscle, as evidenced by immunoblotting and a biosensor [11]. Furthermore, Plk1 phosphorylation is catalyzed by Ste20-like kinase (SLK). Thus, a new
network is involved in focal adhesion dynamics, cell contraction, and protrusion formation [37].

Vimentin intermediate filaments connect with paxillin-positive focal adhesions in the protrusion of motile human airway smooth muscle cells [39]. This raises the possibility that vimentin filaments may regulate focal adhesion dynamics directly and thus cell migration. The interaction of vimentin filaments with focal adhesions is regulated by its phosphorylation at Ser-56, which is coordinated by the miR-509-Plk1 pathway. Exposure of human airway smooth muscle cells to miR-509 reduces the expression of Plk1, which diminishes vimentin phosphorylation at Ser-56 and the connection of vimentin filaments with paxillin. Moreover, focal adhesion size and cell migration are reduced by miR-509. Thus, miR-509 modulates vimentin phosphorylation, vimentin framework reorganization, focal adhesion dynamics, and smooth muscle cell migration by controlling Plk1 expression. Physical connection of vimentin filaments with focal adhesions may promote the activation of focal adhesion kinase and focal adhesion dynamics [40]. Interestingly, the expression of miR-509 is reduced in asthmatic human airway smooth muscle cells. This is encouraging because lower miR-509 expression may contribute to increased airway smooth muscle cell migration, which is involved in airway remodeling in asthma [39].

In addition to phosphorylation, vimentin cytoskeleton is regulated by other post-translational modifications such as glycosylation. Using genetic engineering, Tarbet et al. produced 293T cells with modified vimentin proteins, which lacked site-specific attachment of O-linked β-N-acetylglucosamine (O-GlcNAc). Mutant vimentin protein lost ability to form vimentin filaments and inhibited cell migration [41]. However, future studies are required to assess whether vimentin glycosylation occurs during smooth muscle cell migration.

As described earlier, cell contraction is critical for inducing retraction of the rear. Vimentin phosphorylation at Ser-56 regulates the reorganization of the vimentin network of smooth muscle cells, which may affect the realignment of contractile element and smooth muscle cell migration [15,16,37]. Additionally, vimentin phosphorylation at Ser-56 is important for locomotion of endothelial cells [17] and cancer cells [42].

The vimentin network indirectly regulates lamellipodial formation by affecting the actin cytoskeleton. Vimentin phosphorylation at Ser-56 by Pak1 and Plk1 leads to its partial disassembly in smooth muscle, which results in the release of p130CAS from cytoskeletal vimentin. p130CAS translocates to the cell cortex and promotes the Arp2/3 complex-mediated actin polymerization and branching and lamellipodial formation [1,3,6,11,15,18,43,44]. In addition, caldesmon is a component of thin filaments in smooth muscle cells. Caldesmon interacts with intermediate filaments and polymerized actin and is required for maintaining the intermediate filament network and actin filaments in smooth muscle cells [45]. Caldesmon phosphorylation by the serine/threonine protein kinase PFTAIRE1 promotes its binding to F-actin and stress fiber formation in motile cells [30]. Furthermore, CARMIL2 (capping protein, Arp2/3, myosin-I linker 2) is a molecule that regulates the activity of capping protein. During migration, dynamic vimentin filaments target CARMIL2 to the cell cortex, where CARMIL2 modulates the capping protein activity and increases local actin filament assembly and protrusion formation [46].

Vimentin Intermediate Filaments and Nucleus Positioning

The cytoplasmic vimentin network organizes around the nucleus of smooth muscle cells and maintains adequate nucleus positioning [15,22]. Vimentin filaments connect with the nuclear envelop probably via the envelope proteins forming the linker of the nucleoskeleton to the cytoskeleton (LINC) complex [47]. The IF-associated protein nesprin-3 localizes in the outer nuclear membrane and interacts with IFs via plectin. IFs may also indirectly interact with the nucleus via microtubules or microfilaments.

Role of the Vimentin Network in Cell Migration

Smooth muscle cell migration is a normal process that occurs during the development of the airways and blood vessels. However, smooth muscle cell motility is also associated with the pathogenesis of airway/vascular remodeling [5,37]. Cell migration includes the cycles of the following four steps: lamellipodial formation, focal adhesion assembly in front, cell contraction, and focal adhesion disassembly in rear [18,37,38]. The vimentin network indirectly regulates lamellipodial formation by affecting the actin cytoskeleton. Vimentin phosphorylation at Ser-56 by Pak1 and Plk1 leads to its partial disassembly in smooth muscle, which results in the release of p130CAS from cytoskeletal vimentin. p130CAS translocates to the cell cortex and promotes the Arp2/3 complex-mediated actin polymerization and branching and lamellipodial formation [1,3,6,11,15,18,43,44]. In addition, caldesmon is a component of thin filaments in smooth muscle cells. Caldesmon interacts with intermediate filaments and polymerized actin and is required for maintaining the intermediate filament network and actin filaments in smooth muscle cells [45]. Caldesmon phosphorylation by the serine/threonine protein kinase PFTAIRE1 promotes its binding to F-actin and stress fiber formation in motile cells [30]. Furthermore, CARMIL2 (capping protein, Arp2/3, myosin-I linker 2) is a molecule that regulates the activity of capping protein. During migration, dynamic vimentin filaments target CARMIL2 to the cell cortex, where CARMIL2 modulates the capping protein activity and increases local actin filament assembly and protrusion formation [46].
that associate with the LINC complex. Furthermore, vimentin filaments may directly interact with the intranuclear lamina, as suggested by the interaction of vimentin and lamin B in vitro [47].

The intermediate filaments are involved in coordinating the size and shape of the nucleus, particularly during migration in a three-dimensional environment. Lamins are the type IV intermediate filament proteins that are the major components of the nuclear membrane and largely affect the mechanical property of the nucleus [1,37]. Because vimentin filaments associate with lamin B [47], reorganization of the vimentin network may affect the size and shape of the nucleus of motile cells. Additionally, vimentin expression levels are associated with nuclear rigidity and chromatin remodeling [48]. It is likely that vimentin is involved in the regulation of gene expression as well.

Conclusions and Perspectives

The vimentin network links to the intercellular junction and dense bodies in the cytoplasm, which provides a structural base for intercellular and intracellular force transmission in smooth muscle. The vimentin framework plays an important role in mediating smooth muscle contraction. However, the knowledge regarding the role of vimentin filaments in other airway smooth muscle biophysical properties (e.g., stiffness and traction force) is lacking. The vimentin network undergoes phosphorylation at Ser-56 and reorganization upon contractile stimulation, facilitating mechano-transmission in smooth muscle. The signaling cascades regulating vimentin phosphorylation at Ser-56 have been unveiled in smooth muscle. Vimentin filaments are also involved in cell migration and nuclear positioning; however, the mechanisms by which the vimentin network regulates these processes remain to be elucidated. Moreover, more studies are required to reveal the role of vimentin in smooth muscle cell proliferation, apoptosis, mitosis, and other functions.

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