Current Topics in Biochemical Research
Vol. 16, 2014

Review

Calponin: A mechanical tension-modulated regulator of cytoskeleton and cell motility

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ABSTRACT

Calponin is an actin filament-associated regulatory protein expressed in smooth muscle and multiple types of non-muscle cells. Three isoforms of calponin are identified in vertebrate species. The structure-function relationship of calponin has been extensively investigated in terms of the regulation of smooth muscle contractility, in which its function was established as an inhibitor of actin-activated myosin ATPase. Besides modulating smooth muscle myofilaments, calponin also regulates the function of actin cytoskeleton with effects on cellular activities, such as adhesion, migration, division and differentiation. More recent studies demonstrated that calponin's gene expression and protein turnover are both regulated by mechanical tension in correlation with its function as a stabilizer of the actin cytoskeleton and a regulator of cell motility. This finding also provides a novel system to study the interaction between extracellular mechanical environment and cellular functions. Summarizing recent research progresses, this review focuses on the role of calponin and its isoforms in mediating responses of cytoskeleton and cell motility to mechanical tension with examples for their functions in different cell types.

KEYWORDS: calponin, cytoskeleton, cell motility, adhesion, migration, phagocytosis, mechanoregulation

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ABBREVIATIONS

bFGF, basic fibroblast growth factor; CH domain, calponin homology domain; CMV, cytomegalovirus; ERK, extra-cellular regulated kinase; F-actin, filament actin; GPI, glucose 6 phosphate isomerase; HES-1, hairy and enhancer of split 1; MAPK, mitogen-activated protein kinase; MEK, mitogen activated protein/extracellular signal regulated kinase; NCC, neural crest cell; PC-M, metastatic prostate cancer cell lines; PKC, protein kinase C; RBP, recombining binding protein suppressor of hairless.

INTRODUCTION

Calponin is an actin filament-associated regulatory protein, expressed in smooth muscle and many non-muscle cell types. It has evolved into three isoforms in vertebrate species encoded by three homologous genes: *CNN1*, *CNN2* and *CNN3* [1, 2]. Comparisons of cDNA and the deduced protein sequences of the calponin isoforms demonstrated their highly conserved primary structure, indicating a functional similarity of the three isoforms as actin filament associated regulatory proteins. On the other hand, the three calponin isoforms are differentiated in the C-terminal segment that is a variable region primarily responsible for their structural differences. The differences in charged residues in the C-terminal variable region determine the overall charge of the calponin isoforms: h1-calponin encoded by *CNN1* is basic, h2-calponin encoded by *CNN2* is neutral, and h3-calponin encoded by *CNN3* is acidic [3-6]. The evolutionary lineage among vertebrate calponin isoforms

shown in Fig. 1 reveals a pattern in which each of the isoforms is more conserved among species whereas the three isoforms have been distinctly diverged in all vertebrate species.

The three calponin isoforms exhibit different patterns of cell type-specific expression (Table 1), reflecting their functional diversities corresponding to the specific cellular environment and/or activity in different cell types. H1-calponin is the first calponin identified with abundance in smooth muscle cells [7]. The function of h1-calponin has been extensively studied and characterized with a role in regulating the interaction between actin filament and myosin during smooth muscle contraction [8]. H2-calponin plays a role in regulating cell proliferation and migration via modifying actin cytoskeleton in both smooth muscle and many non-muscle cells, such as fibroblasts, epithelial and endothelial cells. H3- (acidic) calponin is found in the brain, with expression in neurons [9], astrocytes [10], and glial cells [11], in the actin cytoskeleton with a proposed role in the

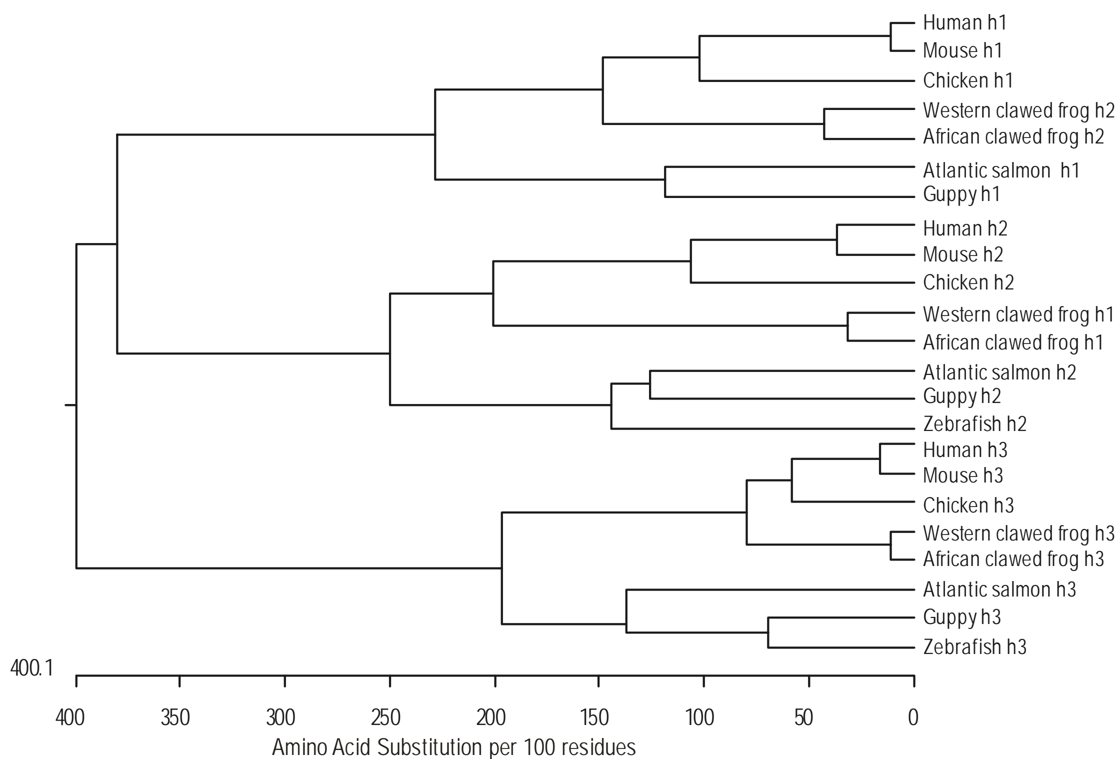

Fig. 1. Evolutionary lineage of calponin isoforms. The phylogenetic tree was constructed using the Jotun Hein method of DNAStar MegAlign program to align the amino acid sequences of representative mammalian, avian, amphibian and fish h1, h2 and h3 isoforms of calponin. The degrees of evolutionary divergence are indicated by the lengths of lineage lines. GenBank/EBI Data Bank accession numbers for the calponin isoforms analyzed are: human h1-calponin, D17408; human h2-calponin, NM_004368; human h3 (acidic) calponin, S80562; mouse h1-calponin, L49022; mouse h2-calponin, Z19543; mouse h3 (acidic) calponin, NM_028044; chicken h1-calponin, M63559; chicken h2-calponin, CD218934 and BG710992; chicken h3 (acidic) calponin, XM_422326; Western clawed frog h1-calponin, NP_998841.1; Western clawed frog h2-calponin, NP_001015796.1; Western clawed frog h3 (acidic) calponin, NP_989257.1; African clawed frog h1-calponin, NP_001080753.1; African clawed frog h2-calponin, NP_001085014.1; African clawed frog h3 (acidic) calponin, NP_001080482.1; Atlantic salmon h1-calponin, NP_001139857.1; Atlantic salmon h2-calponin, NP_001133873.1; Atlantic salmon h3 (acidic) calponin, NP_001133337.1; Guppy h1-calponin, XP_008413565.1; Guppy h2-calponin, XP_008431663.1; Guppy h3 (acidic) calponin, XP_008436465.1; Zebra fish h2-calponin, NP_998514.1; Zebra fish h3 (acidic) calponin NP_001019244.1. It is worth noting that one allele of amphibian *Cnn1* in GenBank is given as *Cnn2*. Therefore, amphibian *Cnn1* and *Cnn2* gene sequences and evolutionary diversity requires further confirmation.

Table 1. Tissue distribution of calponin isoforms.

Tissue or cell type	*CNN1*	*CNN2*	*CNN3*	References
Smooth muscle	+	+	+	[7]
				[3]
				[136]
Neuronal tissue	-	+	+	[9]
				[91]
Fibroblasts	-	+	+	[24]
				[108]
Myoblasts	-	-	+	[14]
Trophoblasts	-	-	+	[15]
Epithelial cells	-	+	+	[23]
				[108]
Endothelial cells	-	+	-	[26]
Macrophages	-	+	-	[27]

Current literature has documented that *CNN1* encoding h1-calponin is specifically expressed in smooth muscle cells; *CNN2* encoding h2-calponin is expressed in both smooth muscle and many non-muscle cells; and *CNN3* encoding acidic calponin is expressed in smooth muscle cells, fibroblast, epithelial cells, neuronal tissues and trophoblast.

plasticity of neural tissues [12, 13]. It is also present in embryonic trophoblasts and myoblasts with functions in cell fusion [14, 15].

Although calponin is an abundant protein present in many cell types, its physiological function is not well established. Based on recent research progress, this focused review summarizes the diversity of calponin isoforms, cell type-specific expression of isoform genes, biochemical properties, the function of calponin in regulating actomyosin interactions, and transcriptional and proteolytic regulations in responses to mechanical tension. Other than broadly outlining all published calponin studies, we have put an emphasis on the novel mechanoregulation of calponin and its functional effects on cell adhesion, migration, proliferation and phagocytosis.

Three calponin isoform genes and their tissue-specific expression

Although amino acids sequence analysis for the evolutionary divergence clearly demonstrated that each of the calponin isoforms is well conserved in the mammalian, avian, amphibian and fish classes, the three isoforms of calponin have significantly diverged among the vertebrate species studied to date (Fig. 1). This feature may reflect adaptations to differentiated cellular functions and tissue environments [1].

The majority of previous structural and functional studies of calponin were obtained from experiments using chicken gizzard calponin. Sequence homology, physical properties, and immunological cross reactivity indicate that chicken gizzard calponin is equivalent to mammalian h1-calponin encoded by *CNN1* gene. The expression of h1-calponin is considered to be specific in differentiated smooth muscle cells and up-regulated during postnatal development [16-18], consistent with a role in contractile function. Nonetheless, h1-calponin has also been found to express in cultured human glomerular mesangial cells [19], myofibroblasts [20] pancreatic precursor cells [21] and Sertoli cells [22]. Although its function in the multiple cell types is not fully understood, it is predicted to relate to the regulation and function of the

actin cytoskeleton. It is interesting that the expression of h1-calponin in pancreatic precursor cells is decreased as the cells differentiate into insulin-producing cells, while over-expression of h1-calponin suppressed cell differentiation [21].

The expression of h2-calponin shows a broad tissue and cell type distribution, including developing and remodeling as well as adult mature smooth muscles [17], epidermal keratinocytes [23], fibroblasts [24], lung alveolar cells [25], endothelial cells [26], and white blood cells of myeloid lineage [27]. These cell types can be classified as cell types that are either physiologically under high mechanical tension (e.g., smooth muscle, epithelial and endothelial cells), with high rates of proliferation (e.g., fibroblasts and myeloid blood cells), and/or active migration (e.g., fibroblasts and macrophages). Therefore, the tissue distribution of h2-calponin implies its potential role in cytoskeleton activities and cell motility, especially cellular interactions with the extracellular mechanical environment.

H3-calponin, also named acidic calponin according to its acidic isoelectric point, is found in smooth muscle [3] and some non-muscle cells [9]. Unlike h1-calponin, h3-calponin has little effect on actomyosin Mg^{2+}-ATPase activity and does not cause actin filaments bundling at the same condition as h1-calponin does [28]. H3-calponin was detected in the brain with a potential function in regulating actin filaments during neuronal remodeling [29]. Recent studies found expression of h3-calponin in embryonic trophoblasts [15] and skeletal muscle myoblasts [14] with an interesting role in cell fusion during embryonic development and myogenesis. Mice with systemic knockout of h1-calponin [30] or h2-calponin [27], or double knockout of h1 and h2 calponins (our unpublished results) survive to adulthood and are fertile. However, systemic knockout of h3-calponin in mice results in prenatal lethality (our unpublished results). Therefore, the function of h3-calponin is apparently critical to embryonic development, which merits further investigation.

Structure and biochemical functions of calponin

Current knowledge of the structure-function relationship of calponin was largely learned from studies on h1-calponin in smooth muscles. Illustrated in Fig. 2, the primary structure of calponin contains an N-terminal calponin homology (CH) domain conserved in all three isoforms and many other proteins, a middle region containing actin-binding sites and a C-terminal variable region. CH domains have been found as tandem repeats in proteins with functions including actin cross-linking to cell signaling and are proposed to be either autonomous actin binding motifs or to serve as regulatory structure [31]. However, the single CH domain in calponin does not determine the modes of calponin binding to F-actin [32]. CH domain of calponin was found to bind to extra-cellular regulated kinase (ERK) 1 and 2, suggesting a function in ERK signaling of smooth muscle and non-muscle cells [33].

A significant amount of calponin was found to co-localize with the cytoskeletal actin filaments [34]. A large collection of evidence illustrated the role of calponin as an actin binding protein that functions as a stabilizer of actin filaments. Calponin binds to actin through two binding sites that are conserved in the three isoforms (Fig. 2). They share common binding regions on actin, amino acid residues 18-28 and 360-372, but only binding to the 18-28 site results in a reduction in F-actin depolymerization, correlative to the fact that the amino acid 18-28 region also determines the release of ATP from actin [35]. Consistently, it induced actin polymerization, caused bundling of actin filaments [36], and inhibited actin-myosin interactions. The C-terminal segment of calponin has an inhibitory effect on association with actin filament by altering the function of the second actin-binding site. Removal of the inhibitory C-terminal segment resulted in increased binding and bundling activities. Domain-swap experiments demonstrated that the C-terminal segment of h2-calponin decreased cytoskeletal association in all three isoforms, whereas the tail segment of h1-calponin had little effect [37].

Biochemical studies demonstrated that h1-calponin inhibits the actin-activated Mg-ATPase of smooth muscle myosin [38, 39, 40] and the movement of actin filaments over immobilized myosin heads [41, 42]. In cultured cells lacking endogenous calponin, transfective expression of h2-calponin resulted in increased resistance of actin filaments to cytochalasin B, indicating increased stability

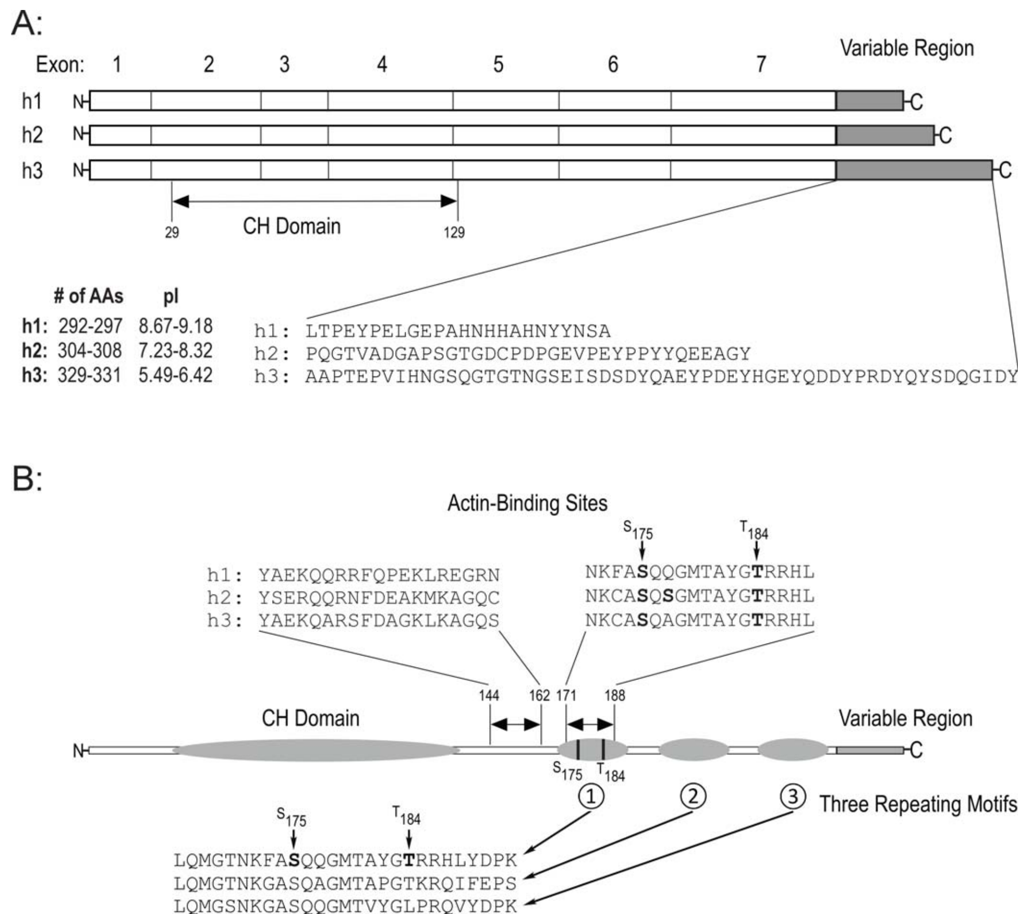

Fig. 2. Structural alignment and comparison of calponin isoforms. (A) This illustration summarizes the primary structure outlines of the three calponin isoforms. The conserved CH domain (residues 29–129 in h1-calponin) is encoded by exons 2, 3 and 4. While the three isoforms are largely conserved in the N-terminal and middle regions, they are significantly different in the C-terminal region. The different length and amino acid sequences of the C-terminal segment determine the size and charge differences of the calponin isoforms. The total number of amino acids (AAs) and isoelectric point of the three isoforms of human, mouse and chicken are presented. (B) The structural map summarized from chicken h1-calponin illustrates the structural and functional domains of calponin. The N terminal CH-domain, the middle region containing two actin-binding sites and three repeating sequence motifs, and the C-terminal variable region are identified. The second actin-binding site and the regulatory phosphorylation site Ser_{175} and Thr_{184} are located in repeat 1 and conserved residues are found in repeats 2 and 3. Amino acid sequences of the two actin-binding sites in the three isoforms and the three repeating motifs of h1-calponin are listed.

of the actin skeleton [24]. H2-calponin co-localizes with tropomyosin in actin stress fibers, contributing to the stabilization effect [24].

In addition to its high binding affinity for F-actin [7, 38] and cross-linking of actin filaments [43], *in vitro* protein binding studies have documented that calponin also binds to tubulin [44, 45], microtubules [46], Ca^{2+}-calmodulin [7, 8], tropomyosin [47, 48], myosin [49, 50], regulatory light chain of myosin [51], desmin [52, 53, 54], caldesmon [55], caltropin [56], gelsolin [57, 58], HSP90 [59, 60], and phospholipids [61, 62].

Three conserved repeating sequence motifs of 27 amino acids each are found in all three isoforms of calponin next to the C-terminal variable region. The first repeating motif contains one of the two actin-binding sites and residues Ser_{175} and Thr_{184} that are phosphorylated by

protein kinase C (PKC) (Fig. 2). Besides *in vitro* phosphorylation studies, direct associations of calponin and PKCα and PKCε have been found in smooth muscle cells [63 64, 65, 66]. Calmodulin-dependent kinase II and Rho-kinase are also found to phosphorylate calponin at Ser_{175} and Thr_{184} *in vitro* [67, 68]. Dephosphorylation of calponin is catalyzed by type 2B protein phosphatase [69, 70]. Phosphorylation of Ser_{175} reduces calponin's inhibition of actomyosin ATPase [71]. This regulatory function is supported by the observation that phosphorylation of Ser_{175} decreases the binding affinity of calponin for F-actin [72]. It has been observed that calponin is transiently phosphorylated in contracting smooth muscle cells [73, 74] and concomitant with the production of tension. This phosphorylation site and flanking sequences are conserved in all three calponin isoforms. Therefore, Ser_{175} may also be a regulatory site in h2- and h3-calponins.

The structure-function relationships of calponin are summarized in Fig. 2. The role of phosphorylation in regulating the physiological function of calponin has been controversial. Despite clear evidence for the phosphorylation regulation of the biochemical function of calponin established in *in vitro* studies, phosphorylated calponin is not readily detectable *in vivo* or in living cells under physiological conditions [75]. Therefore, the cellular dynamics and mechanisms for phosphorylation to regulate the function of calponin remain to be established.

Calponin in smooth muscle cells

H1-calponin is the first calponin isoform identified and abundantly expressed in mature smooth muscle cells [7]. Despite the *in vitro* experimental evidence that suggests a role of calponin in regulating the contractile function of smooth muscle actin filaments [76, 74], the largely normal phenotypes of h1-calponin gene knockout mice demonstrated that the loss of h1-calponin did not abolish the contractility of smooth muscle [30]. This outcome was consistent with earlier observations that vascular smooth muscle of adult Wistar Kyoto rats, which naturally lacks h1-calponin, is contractile although with a decreased sensitivity to norepinephrine activation [77, 78]. This non-essential but regulatory role of h1-calponin was further demonstrated by the observation that matrix metalloproteinase-2 proteolysis of h1-calponin in endotoxemic rats resulted in vascular hypocontractility to phenylephrine [79]. These data indicate that h1-calponin plays a modulator role in the function of smooth muscle actin filaments.

Early notions suggested that h1-calponin functions in smooth muscle thin filament analogously of troponin in striated muscle thin filament by inhibiting actomyosin interactions [7, 80, 81]. More recent studies have suggested that h1-calponin also takes part in stress fiber stabilization and facilitates agonist-induced signal transduction and contractility in smooth muscle by acting as an adaptor protein for ERK1/2 and PKCα/ε in differentiated smooth muscle cells [33, 82, 83, 84]. During force development, the cytoskeletal structure of smooth muscle cells undergoes reorganization, including a loss of actin fibers accompanied with translocation of actin to podosomes [85, 86], an event mediated by PKC [87, 88]. H1-calponin co-localizes with and stabilizes actin stress fiber in resting smooth muscle cells, but disassociates from actin and redistributes to podosome in response to PKC during contraction. Down-regulation of h1-calponin results in both loss in actin stress fiber and decreased response to PKC agonist with cell contraction and podosome formation, indicating that calponin is necessary for the stability of actin cytoskeleton and the disruption of the contractile apparatus blocks subsequent contraction and cytoskeletal remodeling [83].

H2-calponin is also expressed in smooth muscle cells. Significant amounts of h2-calponin are found in growing smooth muscle tissues such as embryonic stomach and urinary bladder as well as uterus during early pregnancy [17]. The expression of h2-calponin decreases to low levels in quiescent adult smooth muscle cells while the expression of h1-calponin increases [17]. Forced expression of h2-calponin inhibited the division and proliferation of smooth muscle cells [17], indicating a function in regulating smooth muscle growth. This observation is consistent with later findings that h2-calponin is expressed at high levels in non-muscle cells with a role in regulating cell proliferation [2]. Taking together, h2-calponin may contribute to different functions in smooth muscle cells from that of h1-calponin by

regulating non-contractile cytoskeleton activities such as cell proliferation and migration in developing and remodeling smooth muscle [17].

H3-calponin is also expressed in smooth muscle cells [3] but the regulation and function remain to be investigated.

Calponin in non-muscle cell motility

Whereas h1-calponin is exclusively expressed in differentiated smooth muscle cells, h2-calponin and h3-calponin are expressed also in multiple non-muscle cell types [1, 2]. H2-calponin has been detected in epithelial cells such as HEK293 cells [89], epidermal keratinocytes [24, 25], endothelial cells [26], fibroblasts [24] and blood cells of myeloid lineage including peripheral monocytes and macrophages [27]. The abundant presence of h2-calponin in such multiple cell types implies that h2-calponin plays a function that is common in smooth muscle and a broad range of non-muscle cells to regulate activities of the actin cytoskeleton.

The dynamics of actin cytoskeleton plays a critical role in cell motility-based functions, such as cell adhesion, migration, proliferation, and phagocytosis. Rearrangement of actin filaments is essential for cells to maintain shape, to migrate, to divide, to secrete cellular products and to engulf objects during phagocytosis [90]. As an actin filament-associated regulatory protein, h2-calponin stabilizes the actin skeleton as a negative regulator to balance motility-based cellular activities.

An *in vivo* study found that h2-calponin mRNA is present in protrusions at the leading edge of migratory neural crest cell (NCC). As actin-driven formation of polarized cell protrusions is important for NCCs migration, *Cnn2* knockdown resulted in NCC randomized outgrowth of protrusions and migration defects, accompanied with central stress fiber formation with reduced peripheral actin network [91].

More studies have investigated the function of calponin in regulating the actin-cytoskeleton in cell types with different biological features [24-27]. For example, a study on the role of h2-calponin in macrophages [27] showed a higher rate of proliferation and faster migration of h2-calponin-null mouse macrophages than that of h2- calponin-positive wild type cells. Moreover, the knockout of h2-calponin gene significantly increased macrophage phagocytotic activity. The results were consistent with earlier biochemical studies showing that calponin functions as an inhibitor of actomyosin interaction [67]. The results indicate that h2-calponin is an inhibitor of cell proliferation, migration and phagocytosis, suggesting a novel mechanism to modulate the function of macrophages in immune responses and the treatment of inflammatory diseases.

Interestingly, another study showed that h2-calponin played variable roles in cell motility in endothelial cells, in which force-expression of h2-calponin enhanced cell migration and anti-sense h2-calponin RNA reduced chemotaxis of human umbilical vein endothelial cells in culture [26]. The observation that calponin expression was positively related to the degree of cytoskeleton activity is opposite to the inhibitory effects on cell motility in many other cell types studied. A hypothesis is that a proper level of h2-calponin might be critical to maintain the physiological balance of cell motility and different cell types may have different internal set points, in which the requirement for the balancing activity of calponin is different. This hypothesis is worth further investigation.

H2-calponin in cellular mechanoregulation

With a primary function in stabilizing actin cytoskeleton, the expression of h2-calponin gene and the degradation of h2-calponin protein are both regulated by mechanical tension built in the cytoskeleton [24, 25]. There are multiple experimental evidences that support this hypothesis: The expression of h2-calponin was significantly increased in cells cultured on high versus low stiffness substrates that produce high or low traction force and cytoskeleton tension in the cells [24]. This regulation is demonstrated at both protein and mRNA levels [25]. The expression of h2-calponin in NIH/3T3 cells was decreased when cytoskeleton tension was reduced after blebbistatin inhibition of the myosin II motors [25].

At the protein level, a rapid and selective degradation of h2-calponin occurs in lung tissues after a short period of deflation [25]. This low cytoskeleton tension-induced degradation of h2-calponin in collapsed lung could be prevented by air inflation to maintain tension applied to the

alveolae in post mortem mouse lung [25]. The tension-dependent stability of h2-calponin was further confirmed in monolayer cells cultured on expanded elastic membrane by its rapid degradation after a reduction of the dimension of the cultural substrate to acutely reduce cytoskeleton tension [25].

To further demonstrate the mechanical tension regulation of h2-calponin gene expression, transfective expression of h2-calponin using a cytomegalovirus promoter behaved independently of the stiffness of culture substrate [24]. This result suggested that a *Cnn2* promoter-specific transcriptional regulation is the primary determinant for the regulation of h2-calponin expression in response to mechanical signals. Our recent study investigated the regulatory elements in the 5'-upstream region of mouse *Cnn2* gene promoter for their roles in the responses to substrate stiffness [89]. The results revealed that a binding site for transcriptional factor HES-1 (hairy and enhancer of split 1) mediates a low tension-induced repression of the transcription of *Cnn2* gene. Deletion or mutation of the HES-1 site in *Cnn2* promoter abolished the mechanical regulation and resulted in a substrate stiffness independent high level of transcriptional activity [89]. Corresponding to the down-regulation of h2-calponin, the level of HES-1 increased in cells cultured on soft gel substrates in comparison with that in cells cultured on hard gels [89]. HES-1 is known to function downstream of the Notch-RBP J signaling pathway [92], which has been suggested to mediate cellular mechanoregulations [93, 94]. Consistently, Notch inhibitor increased, whereas Notch activator decreased, h2-calponin expression in a mechanical tension-dependent manner [89].

The mechanical tension-regulated expression of *Cnn2* gene and the function of h2-calponin in stabilizing actin cytoskeleton present a novel system to study cellular mechanoregulation. When sensing mechanical stimuli, the cytoskeleton, as a dynamic tensegrity network, rearranges and redistributes to reduce the overall stress [95]. At the same time, the force signal is transmitted into the nuclei to modulate adaptive gene expressions [96]. In this regulation, the level of h2-calponin gene expression is high at high cytoskeleton tension to make actin cytoskeleton more stable and vice versa. At the post-transcriptional level, h2-calponin is down-regulated by low tension-induced specific proteolysis [25], which decreases F-actin stability and in turn decreases the tension signal transduced into and sensed by the cell, resulting in a repression of h2-calponin gene transcription to further maintain equilibrium of a low level of h2-calponin in the cell. Adding to this hypothesis, recent studies showed that binding of calponin confers increased flexibility of actin filaments, allowing actin networks to withstand a higher maximal strain before failing [97, 98].

Regulation and function of h2-calponin in specific cell types

With a universal role in stabilizing actin cytoskeleton under mechanoregulation, h2-calponin is found in many different cell types. Therefore, h2-calponin may participate in diverse motility-related cellular functions as a mechanoregulatory protein. Experimental evidence has been obtained in the following cell types with different biological features and mechanical tension environments.

H2-calponin in smooth muscle cells

H1-calponin is abundantly expressed in quiescent adult smooth muscle with a function in the fine-tuning of smooth muscle contractility [1]. But in growing smooth muscle tissues, the expression of h1-calponin was lower, which is accompanied by significant levels of h2-calponin expression. The high level expression of h2-calponin in developing and growing smooth muscles suggests a function of h2-calponin in smooth muscle cell proliferation.

Over-expression of h2-calponin inhibits the rate of cell proliferation [17]. The over-expressed h2-calponin was concentrated around dividing nucleus co-localizing with actin stress fibers. This phenotype corresponds to a significant increase in the number of bi-nuclei cells indicating an inhibition of cytokinesis [17]. As smooth muscle cell is a cell type that retains remarkable plasticity [99], the function of h2-calponin in regulating cytoskeleton function during cell division presents a potentially novel mechanism of controlling smooth muscle cell proliferation.

It is well established that vascular smooth muscle cells switch phenotype from contractile to synthetic during vascular remodeling such as that in the pathogenesis of atherosclerosis. Synthetic

smooth muscle cells secrete cytokines and express cell adhesion molecules during inflammatory processes [100], migrate into necrotic lesions, proliferate and form atherosclerosis plaques [101]. Through inhibiting smooth muscle cell proliferation and migration, h2-calponin may play a role in regulating the development and progression of atherosclerosis and other pathological conditions involving vascular remodeling. On the other hand, the function of h2-calponin in smooth muscle cell proliferation, migration and differentiation is not fully understood and more detailed studies are needed.

H2-calponin in fibroblasts

A significant level of h2-calponin is found in fibroblasts [24]. H2-calponin inhibits fibroblast motility and thus is potentially a potent regulator in fibroblast functions. Fibroblast is an essential cell type involved in tissue remodeling. Taking skin wound healing process as an example, residential fibroblasts in the dermis migrate into damaged tissue space, proliferate and excrete matrix molecules to form granulation [102]. Fibroblasts in normal connective tissue do not contain actin stress fibers [103, 104]. When fibroblasts enter the wound space, they experience mechanical tension, which causes them to assemble stress fibers, *i.e.,* bundles of polymerized actin filaments [105, 106]. The anchoring of fibroblasts to culture dish, which is a high stiffness substrate, builds up high intracellular tension, and induces h2-calponin expression, whereas h2-calponin subsequently increases the stability of stress fiber fibroblasts [24]. The development of stress fibers in fibroblasts and the cellular interaction with extracellular matrix are essential in wound repair and closure [107, 108]. Therefore, it is logical to speculate that mechanical tension-regulated expression of h2-calponin plays a role in the functions of fibroblasts during tissue repair and remodeling via effecting on the stability and function of actin filaments.

H2-calponin in macrophages and inflammation

Macrophages play major roles in inflammatory processes, such as wound healing, inflammatory bowel disease, rheumatoid arthritis, and arterial atherosclerosis [109-111]. Migrating into the affected tissue environment, macrophages differentiate into the M1 phenotype during the early stage of inflammation and secrete a variety of inflammatory mediators that promote inflammatory responses. Later, the phenotype of macrophages shifts to the M2 type that produces anti-inflammatory cytokines and facilitates the resolution of inflammation and tissue repair [112]. Phagocytosis, a key function of macrophages and possibly an activity inducing the M2 phenotype, is crucial to the homeostatic clearance of dead and apoptotic cells and other inflammatory materials. Promoting phagocytosis function is considered an active therapeutic approach to resolving inflammation [113, 114]. The deletion of h2-calponin in macrophages of *Cnn2* gene knockout mice resulted in more dynamic actin cytoskeleton, consistent with increased macrophage migration and phagocytosis, which are features of the M2 phenotype [27]. This effect provides a novel mechanism to control macrophage function via regulating the expression level and/or activity of h2-calponin.

It has been demonstrated that in an anti-glucose-6-phosphate isomerase (GPI) serum-induced inflammatory arthritis mouse model, the removal of h2-calponin in macrophages significantly attenuated the severity of inflammation (our unpublished results). The enhanced migration and phagocytotic capacity of macrophages may facilitate the clearance of autoimmune complexes as well as promote M1 to M2 phenotype transition, leading to the resolution of inflammation. Based on the observed beneficial effects of h2-calponin-null macrophages on attenuating the development of inflammatory arthritis, modulation of h2-calponin expression and/or function in macrophages may be explored for applications in the prevention and treatment of other inflammatory diseases.

H2-calponin in cytoskeleton stability and cell adhesion-junction integrity

H2-calponin expresses at significant levels in epithelial cells, for example epidermal keratinocytes and lung alveolar cells [24, 25]. The expression of h2-calponin gene in these cell types is positively dependent on cell adhesion to culture substrate and substrate stiffness. In the mean time, decreases in cellular tension induce significant and selective degradation of h2-calponin protein in lung

alveolar cells [25]. With the demonstrated role of h2-calponin in stabilizing the actin cytoskeleton [24] and cell adhesion [115], h2-calponin may play regulatory functions in epithelial cell adhesion-junction integrity, a hypothesis that is worth investigating.

Different from the effects observed in fibroblasts, epithelial cells and macrophages, knockdown of h2-calponin gene expression down-regulated the migration of endothelial cells, whereas over-expression of h2-calponin enhanced endothelial cells migration and subsequently vascular development, and facilitated wound healing [26]. It is known that MAPK/ERK signaling pathway plays a role in the regulation of endothelial cell migration [116, 117], which is crucial in basic fibroblast growth factor (bFGF)-stimulated endothelial cell migration [118]. Lowering h2-calponin expression in endothelial cells caused a blunting of bFGF-stimulated endothelial cell migration, suggesting that h2-calponin in endothelial cells is involved in MAPK signaling via the function of bFGF. H2-calponin may also participate in MAPK pathways upstream of or at the MEK1/2 level, as observed in MEK1/2 inhibitor studies [26]. The observation of h2-calponin's role in facilitating rather than inhibiting endothelial cell migration leads to a hypothesis that an optimal level of h2-calponin activity for balancing cell motility versus cytoskeleton stability may vary among different cell types.

While endothelial cell migration is a critical function in many physiological and pathological processes involving angiogenesis, formation of adhesion junctions is essential for endothelial cells to maintain structural integrity of the vessel wall as a physiological barrier. Impairment of endothelial cell junctions occurs in many inflammatory processes. Extensive reorganization of the actin cytoskeleton is required during early stages of adhesion junction formation [119], and stabilizing actin cytoskeleton might be beneficial in maintaining the structure of adhesion junctions. Blood shear stress is considered an initial factor for endothelial dysfunction and the pathogenesis of atherosclerosis. As h2-calponin expression level is regulated by outside-in mechanical tension signals and h2-calponin is capable of changing cellular activities by modulating cytoskeleton functions, the role of endothelial h2-calponin in the pathogenesis of atherosclerosis is worth investigating.

Diminished expression of calponin in malignant cells and the effect of mechanical environment on cancer metastasis

Tumor cells derived from various calponin-positive cell types, *e.g.*, carcinomas originating from epithelial cells, showed a unanimous phenotype of decreased expression of calponin. For example, calponin expression is significantly lower in leiomyosarcoma cells than that in normal smooth muscle cells [120]. Reduced levels of h1-calponin were found in hepatocellular carcinoma [121], renal angiomyolipoma [122], mammary simple carcinomas [123], papillary carcinomas [124], basal cell-like breast carcinoma [125], metastatic basal cell carcinomas [126], and prostate cancer [127, 128, 129].

Tumor metastasis requires that tumor cells migrate from the primary site, escape from the vasculature, invade and form secondary tumors at novel sites. These processes depend to a large degree on cytoskeletal remodeling. Reduced levels of calponin expression correlate with alterations in actin cytoskeleton stability in tumor cells [120]. The level of h1-calponin in human osteosarcoma cells is positively correlated with the prognosis [130]. H1-calponin knockout mice had an early onset of cartilage formation and ossification and accelerated healing of bone fractures [131], suggesting that calponin may inhibit tumor cell invasion and growth. Transfective expression of calponin in human fibrosarcoma, leiomyosarcoma, synovial sarcoma and osteosarcoma cells significantly reduced anchorage-independent growth and *in vivo* tumorigenicity, which supports its function as a tumor suppressor [132, 133, 134]. We recently demonstrated that a metastatic prostate cancer cell line PC3-M showed much lower level of h2-calponin than its parental cell line PC3, corresponding to faster rates of cell proliferation and migration. Substrate adhesion of PC3 and PC3-M cells was positively correlated to the level of h2-calponin and the adhesion of PC3-M exhibited a higher dependence on substrate stiffness. The effects of h2-calponin on cell proliferation, migration and substrate adhesion were also seen in

normal versus cancerous primary prostate cells. Transfective over-expression of h2-calponin in PC3-M cells effectively inhibited cell proliferation and migration. The results suggest that the diminished expression of h2-calponin in prostate cancer cells increases cell motility, decreases substrate adhesion, and promotes adhesion on high stiffness substrates [115].

It is worth noting that we recently reported that h2-calponin is potentially a serum marker for the early detection of breast cancer [135], opening a novel field of translational calponin research.

CONCLUSION

In summary, three isoforms of calponin are encoded by three separate genes in vertebrates with tissue-specific expressions in smooth muscle and non-muscle cells to function as regulators of the actin cytoskeleton. Mechanical tension sensitive expression of h2-calponin gene provides a unique model for investigating the molecular mechanisms of cellular regulation by mechanical force. Understanding the relationships between mechanical tension and the dynamics as well as stability of actin cytoskeleton will lay a foundation for the development of novel approaches to control a broad range of cell motility-based functions from smooth muscle contraction to cell migration during development and tissue remodeling to inflammatory process to myogenesis and cancer metastasis. Whereas calponin's function may differ in different cell types, the regulation of calponin function at their gene expression, posttranslational modification and degradation provides attractive molecular targets for controlling actin cytoskeleton activity and cell motility related phenotypes. The selective targeting at an effector molecule like calponin to selectively effect on cell motility-based functions and dysfunctions would be a novel approach to the development of therapeutic strategies for wound healing, chronic autoimmune diseases, atherosclerosis, and cancer metastasis. Therefore, further investigations are merited in order to better understand the specific function and regulation of calponin isoforms in various cell types.

ACKNOWLEDGEMENT

Our calponin research in the past two decades was supported by grants from the Medical Research Council of Canada, Alberta Cancer Board, American Heart Association, March of Dimes, Midwest Eye Bank, and National Institutes of Health (HL086720) to JPJ.

CONFLICT OF INTEREST STATEMENT

The authors have no financial or other conflict of interest to declare regarding this review article.

REFERENCES

1. Jin, J. P., Zhang, Z. and Bautista, J. A. 2008, Crit. Rev. Eukaryot. Gene Expr., 18, 93.
2. Wu, K. C. and Jin, J. P. 2008, Cell Biochem. Biophys., 52, 139.
3. Applegate, D., Feng, W., Green, R. S. and Taubman, M. B. 1994, J. Biol. Chem., 269, 10683.
4. Jin, J. P., Wu, D., Gao, J., Nigam, R. and Kwong, S. 2003, Protein Expr. Purif., 31, 231.
5. Strasser, P., Gimona, M., Moessler, H., Herzog, M. and Small, J. V. 1993, FEBS Lett., 330, 13.
6. Gao, J., Hwang, J. M. and Jin, J. P. 1996, Biochem. Biophys. Res. Commun., 218, 292.
7. Takahashi, K., Hiwada, K. and Kokubu, T. 1986, Biochem. Biophys. Res. Commun., 141, 20.
8. Takahashi, K., Hiwada, K. and Kokubu, T. 1988, Hypertension, 11, 620.
9. Trabelsi-Terzidis, H., Fattoum, A., Represa, A., Dessi, F., Ben-Ari, Y. and Der Terrossian, E. 1995, Biochem. J., 306 (Pt 1), 211.
10. Agassandian, C., Plantier, M., Fattoum, A., Represa, A. and Der Terrossian, E. 2000, Brain Res., 887, 444.
11. Ferhat, L., Charton, G., Represa, A., Ben-Ari, Y., der Terrossian, E. and Khrestchatisky, M. 1996, Eur. J. Neurosci., 8, 1501.
12. Ferhat, L., Esclapez, M., Represa, A., Fattoum, A., Shirao, T. and Ben-Ari, Y. 2003, Hippocampus, 13, 845.
13. Plantier, M., Fattoum, A., Menn, B., Ben-Ari, Y., Der Terrossian, E. and Represa, A. 1999, Eur. J. Neurosci., 11, 2801.
14. Shibukawa, Y., Yamazaki, N., Daimon, E. and Wada, Y. 2013, Exp. Cell Res., 319, 633.
15. Shibukawa, Y., Yamazaki, N., Kumasawa, K., Daimon, E., Tajiri, M., Okada, Y., Ikawa, M. and Wada, Y. 2010, Mol. Biol. Cell, 21, 3973.

16. Draeger, A., Gimona, M., Stuckert, A., Celis, J. E. and Small, J. V. 1991, FEBS Lett., 291, 24.
17. Hossain, M. M., Hwang, D. Y., Huang, Q. Q., Sasaki, Y. and Jin, J. P. 2003, Am. J. Physiol. Cell Physiol., 284, C156.
18. Jin, J. P., Walsh, M. P., Resek, M. E. and McMartin, G. A. 1996, Biochem. Cell Biol., 74, 187.
19. Sugenoya, Y., Yoshimura, A., Yamamura, H., Inui, K., Morita, H., Yamabe, H., Ueki, N., Ideura, T. and Takahashi, K. 2002, J. Am. Soc. Nephrol., 13, 322.
20. Lee, S. Y., Choi, J. Y., Jin, D. C., Kim, J. and Cha, J. H. 2010, Anat. Cell Biol., 43, 132.
21. Morioka, T., Koyama, H., Yamamura, H., Tanaka, S., Fukumoto, S., Emoto, M., Mizuguchi, H., Hayakawa, T., Kojima, I., Takahashi, K. and Nishizawa, Y. 2003, Diabetes, 52, 760.
22. Zhu, Q., Emanuele, N. V. and van Thiel, D. H. 2004, Cell Tissue Res., 316, 243.
23. Fukui, Y., Masuda, H., Takagi, M., Takahashi, K. and Kiyokane, K. 1997, J. Dermatol. Sci., 14, 29.
24. Hossain, M. M., Crish, J. F., Eckert, R. L., Lin, J. J. and Jin, J. P. 2005, J. Biol. Chem., 280, 42442.
25. Hossain, M. M., Smith, P. G., Wu, K. and Jin, J. P. 2006, Biochemistry, 45, 15670.
26. Tang, J., Hu, G., Hanai, J., Yadlapalli, G., Lin, Y., Zhang, B., Galloway, J., Bahary, N., Sinha, S., Thisse, B., Thisse, C., Jin, J. P., Zon, L. I. and Sukhatme, V. P. 2006, J. Biol. Chem., 281, 6664.
27. Huang, Q. Q., Hossain, M. M., Wu, K., Parai, K., Pope, R. M. and Jin, J. P. 2008, J. Biol. Chem., 283, 25887.
28. Fujii, T., Yabe, S., Nakamura, K. and Koizumi, Y. 2002, Biol. Pharm. Bull., 25, 573.
29. Rami, G., Caillard, O., Medina, I., Pellegrino, C., Fattoum, A., Ben-Ari, Y. and Ferhat, L. 2006, Hippocampus, 16, 183.
30. Takahashi, K., Yoshimoto, R., Fuchibe, K., Fujishige, A., Mitsui-Saito, M., Hori, M., Ozaki, H., Yamamura, H., Awata, N., Taniguchi, S., Katsuki, M., Tsuchiya, T. and Karaki, H. 2000, Biochem. Biophys. Res. Commun., 279, 150.
31. Gimona, M., Djinovic-Carugo, K., Kranewitter, W. J. and Winder, S. J. 2002, FEBS Lett., 513, 98.
32. Galkin, V. E., Orlova, A., Fattoum, A., Walsh, M. P. and Egelman, E. H. 2006, J. Mol. Biol., 359, 478.
33. Leinweber, B. D., Leavis, P. C., Grabarek, Z., Wang, C. L. and Morgan, K. G. 1999, Biochem. J., 344 (Pt 1), 117.
34. North, A. J., Gimona, M., Cross, R. A. and Small, J. V. 1994, J. Cell Sci., 107 (Pt 3), 437.
35. Ferjani, I., Fattoum, A., Manai, M., Benyamin, Y., Roustan, C. and Maciver, S. K. 2010, Biochim. Biophys. Acta, 1804, 1760.
36. Kolakowski, J., Makuch, R., Stepkowski, D. and Dabrowska, R. 1995, Biochemical Journal, 306, 199.
37. Burgstaller, G., Kranewitter, W. J. and Gimona, M. 2002, J. Cell Sci., 115, 2021.
38. Winder, S. J. and Walsh, M. P. 1990, J. Biol. Chem., 265, 10148.
39. Abe, M., Takahashi, K. and Hiwada, K. 1990, J. Biochem., 108, 835.
40. Winder, S. J., Walsh, M. P., Vasulka, C. and Johnson, J. D. 1993, Biochemistry, 32, 13327.
41. Shirinsky, V. P., Biryukov, K. G., Hettasch, J. M. and Sellers, J. R. 1992, J. Biol. Chem., 267, 15886.
42. Haeberle, J. R. 1994, J. Biol. Chem., 269, 12424.
43. Leinweber, B., Tang, J. X., Stafford, W. F. and Chalovich, J. M. 1999, Biophys. J., 77, 3208.
44. Fujii, T., Hiromori, T., Hamamoto, M. and Suzuki, T. 1997, J. Biochem., 122, 344.
45. Fujii, T. and Koizumi, Y. 1999, J. Biochem., 125, 869.
46. Fattoum, A., Roustan, C., Smyczynski, C., Der Terrossian, E. and Kassab, R. 2003, Biochemistry, 42, 1274.
47. Takahashi, K., Hiwada, K. and Kokubu, T. 1987, Life Sci., 41, 291.
48. Childs, T. J., Watson, M. H., Novy, R. E., Lin, J. J. and Mak, A. S. 1992, Biochim. Biophys. Acta, 1121, 41.
49. Lin, Y., Ye, L. H., Ishikawa, R., Fujita, K. and Kohama, K. 1993, J. Biochem., 113, 643.

50. Szymanski, P. T. and Tao, T. 1993, FEBS Lett., 334, 379.
51. Szymanski, P. T. and Goyal, R. K. 1999, Biochemistry, 38, 3778.
52. Fujii, T., Takagi, H., Arimoto, M., Ootani, H. and Ueeda, T. 2000, J. Biochem., 127, 457.
53. Mabuchi, K., Li, B., Ip, W. and Tao, T. 1997, J. Biol. Chem., 272, 22662.
54. Wang, P. and Gusev, N. B. 1996, FEBS Lett., 392, 255.
55. Graceffa, P., Adam, L. P. and Morgan, K. G. 1996, J. Biol. Chem., 271, 30336.
56. Wills, F. L., McCubbin, W. D. and Kay, C. M. 1994, Biochemistry, 33, 5562.
57. Ferjani, I., Fattoum, A., Maciver, S. K., Benistant, C., Chahinian, A., Manai, M., Benyamin, Y. and Roustan, C. 2006, Biochem. J., 396, 461.
58. Ferjani, I., Fattoum, A., Maciver, S. K., Manai, M., Benyamin, Y. and Roustan, C. 2007, Biochim. Biophys. Acta, 1774, 952.
59. Bogatcheva, N. V., Ma, Y., Urosev, D. and Gusev, N. B. 1999, FEBS Lett., 457, 369.
60. Ma, Y., Bogatcheva, N. V. and Gusev, N. B. 2000, Biochim. Biophys. Acta, 1476, 300.
61. Bogatcheva, N. V. and Gusev, N. B. 1995, FEBS Lett., 371, 123.
62. Fujii, T., Yamana, K., Ogoma, Y. and Kondo, Y. 1995, J. Biochem., 117, 999.
63. Somara, S. and Bitar, K. N. 2008, Am. J. Physiol. Gastrointest. Liver Physiol., 295, G1246.
64. Patil, S. B., Pawar, M. D. and Bitar, K. N. 2004, Am. J. Physiol. Gastrointest. Liver Physiol., 286, G954.
65. Leinweber, B., Parissenti, A. M., Gallant, C., Gangopadhyay, S. S., Kirwan-Rhude, A., Leavis, P. C. and Morgan, K. G. 2000, J. Biol. Chem., 275, 40329.
66. Naka, M., Kureishi, Y., Muroga, Y., Takahashi, K., Ito, M. and Tanaka, T. 1990, Biochem. Biophys. Res. Commun., 171, 933.
67. Walsh, M. P. 1991, Biochem. Cell Biol., 69, 771.
68. Kaneko, T., Amano, M., Maeda, A., Goto, H., Takahashi, K., Ito, M. and Kaibuchi, K. 2000, Biochem. Biophys. Res. Commun., 273, 110.
69. Fraser, E. D. and Walsh, M. P. 1995, Biochemistry, 34, 9151.
70. Ichikawa, K., Ito, M., Okubo, S., Konishi, T., Nakano, T., Mino, T., Nakamura, F., Naka, M. and Tanaka, T. 1993, Biochem. Biophys. Res. Commun., 193, 827.
71. Tang, D. C., Kang, H. M., Jin, J. P., Fraser, E. D. and Walsh, M. P. 1996, J. Biol. Chem., 271, 8605.
72. Jin, J. P., Walsh, M. P., Sutherland, C. and Chen, W. 2000, Biochem. J., 350 (Pt 2), 579.
73. Winder, S. J. and Walsh, M. P. 1993, Cell Signal., 5, 677.
74. Gerthoffer, W. T. and Pohl, J. 1994, Can. J. Physiol. Pharmacol., 72, 1410.
75. Gimona, M., Sparrow, M. P., Strasser, P., Herzog, M. and Small, J. V. 1992, Eur. J. Biochem., 205, 1067.
76. Winder, S. J., Allen, B. G., Clement-Chomienne, O. and Walsh, M. P. 1998, Acta Physiol. Scand., 164, 415.
77. Nigam, R., Triggle, C. R. and Jin, J. P. 1998, J. Muscle Res. Cell Motil., 19, 695.
78. Facemire, C., Brozovich, F. V. and Jin, J. P. 2000, J. Muscle Res. Cell Motil., 21, 367.
79. Castro, M. M., Cena, J., Cho, W. J., Walsh, M. P. and Schulz, R. 2012, Arterioscler. Thromb. Vasc. Biol., 32, 662.
80. Allen, B. G. and Walsh, M. P. 1994, Trends Biochem. Sci., 19, 362.
81. Takahashi, K., Abe, M., Hiwada, K. and Kokubu, T. 1988, J. Hypertens. Suppl., 6, S40.
82. Menice, C. B., Hulvershorn, J., Adam, L. P., Wang, C. A. and Morgan, K. G. 1997, J. Biol. Chem., 272, 25157.
83. Dykes, A. C. and Wright, G. L. 2007, Can. J. Physiol. Pharmacol., 85, 225.
84. Je, H. D., Gangopadhyay, S. S., Ashworth, T. D. and Morgan, K. G. 2001, J. Physiol., 537, 567.
85. Fultz, M. E., Li, C., Geng, W. and Wright, G. L. 2000, J. Muscle Res. Cell Motil., 21, 775.
86. Black, J., Dykes, A., Thatcher, S., Brown, D., Bryda, E. C. and Wright, G. L. 2009, Can. J. Physiol. Pharmacol., 87, 327.
87. Hai, C. M., Hahne, P., Harrington, E. O. and Gimona, M. 2002, Exp. Cell Res., 280, 64.
88. Gatesman, A., Walker, V. G., Baisden, J. M., Weed, S. A. and Flynn, D. C. 2004, Mol. Cell Biol., 24, 7578.

89. Jiang, W. R., Cady, G., Hossain, M. M., Huang, Q. Q., Wang, X. and Jin, J. P. 2014, J. Biol. Chem., 289, 1617.
90. Carpenter, C. L. 2000, Crit. Care Med., 28, N94.
91. Ulmer, B., Hagenlocher, C., Schmalholz, S., Kurz, S., Schweickert, A., Kohl, A., Roth, L., Sela-Donenfeld, D. and Blum, M. 2013, Cell Rep., 3, 615.
92. Kageyama, R., Ishibashi, M., Takebayashi, K. and Tomita, K. 1997, Int. J. Biochem. Cell Biol., 29, 1389.
93. Morrow, D., Sweeney, C., Birney, Y. A., Cummins, P. M., Walls, D., Redmond, E. M. and Cahill, P. A. 2005, Circ. Res., 96, 567.
94. Morrow, D., Sweeney, C., Birney, Y. A., Guha, S., Collins, N., Cummins, P. M., Murphy, R., Walls, D., Redmond, E. M. and Cahill, P. A. 2007, Am. J. Physiol. Cell Physiol., 292, C488.
95. Kang, J., Steward, R. L., Kim, Y., Schwartz, R. S., LeDuc, P. R. and Puskar, K. M. 2011, J. Theor. Biol., 274, 109.
96. Shivashankar, G. V. 2011, Annu. Rev. Biophys., 40, 361.
97. Jensen, M. H., Morris, E. J., Gallant, C. M., Morgan, K. G., Weitz, D. A. and Moore, J. R. 2014, Biophys. J., 106, 793.
98. Jensen, M. H., Watt, J., Hodgkinson, J. L., Gallant, C., Appel, S., El-Mezgueldi, M., Angelini, T. E., Morgan, K. G., Lehman, W. and Moore, J. R. 2012, Cytoskeleton (Hoboken), 69, 49.
99. Gomez, D. and Owens, G. K. 2012, Cardiovasc. Res., 95, 156.
100. Orr, A. W., Hastings, N. E., Blackman, B. R. and Wamhoff, B. R. 2010, J. Vasc. Res., 47, 168.
101. Lim, S. and Park, S. 2014, BMB Rep., 47, 1.
102. Barisic-Dujmovic, T., Boban, I. and Clark, S. H. 2010, J. Cell Physiol., 222, 703.
103. Byers, H. R., White, G. E. and Fujiwara, K. 1984, Cell Muscle Motil., 5, 83.
104. Ehrlich, H. P. and Hembry, R. M. 1984, Am. J. Pathol., 117, 218.
105. Hinz, B., Celetta, G., Tomasek, J. J., Gabbiani, G. and Chaponnier, C. 2001, Mol. Biol. Cell., 12, 2730.
106. Tomasek, J. J., Gabbiani, G., Hinz, B., Chaponnier, C. and Brown, R. A. 2002, Nat. Rev. Mol. Cell Biol., 3, 349.
107. Martinez-Ferrer, M., Afshar-Sherif, A. R., Uwamariya, C., de Crombrugghe, B., Davidson, J. M. and Bhowmick, N. A. 2010, Am. J. Pathol., 176, 98.
108. Daimon, E., Shibukawa, Y. and Wada, Y. 2013, Arch. Dermatol. Res., 305, 571.
109. Murray, P. J. and Wynn, T. A. 2011, Nat. Rev. Immunol., 11, 723.
110. Hansson, G. K. and Hermansson, A. 2011, Nat. Immunol., 12, 204.
111. Kamada, N., Hisamatsu, T., Okamoto, S., Chinen, H., Kobayashi, T., Sato, T., Sakuraba, A., Kitazume, M. T., Sugita, A., Koganei, K., Akagawa, K. S. and Hibi, T. 2008, J. Clin. Invest., 118, 2269.
112. Wynn, T. A., Chawla, A. and Pollard, J. W. 2013, Nature, 496, 445.
113. Liu, G., Wu, C., Wu, Y. and Zhao, Y. 2006, Scand. J. Immunol., 64, 1.
114. Silva, M. T. 2011, J. Leukoc. Biol., 89, 675.
115. Hossain, M. M., Wang, X., Bergan, R. C. and Jin, J. P. 2014, FEBS Open Bio., 4, 627.
116. Klemke, R. L., Cai, S., Giannini, A. L., Gallagher, P. J., de Lanerolle, P. and Cheresh, D. A. 1997, J. Cell Biol., 137, 481.
117. Lai, C. F., Chaudhary, L., Fausto, A., Halstead, L. R., Ory, D. S., Avioli, L. V. and Cheng, S. L. 2001, J. Biol. Chem., 276, 14443.
118. Sakihara, C., Nishimura, J., Kobayashi, S., Takahashi, S. and Kanaide, H. 1996, Biochem. Biophys. Res. Commun., 222, 195.
119. Hoelzle, M. K. and Svitkina, T. 2012, Mol. Biol. Cell, 23, 310.
120. Horiuchi, A., Nikaido, T., Ito, K., Zhai, Y., Orii, A., Taniguchi, S., Toki, T. and Fujii, S. 1998, Lab. Invest., 78, 839.
121. Sasaki, Y., Yamamura, H., Kawakami, Y., Yamada, T., Hiratsuka, M., Kameyama, M., Ohigashi, H., Ishikawa, O., Imaoka, S., Ishiguro, S. and Takahashi, K. 2002, Cancer, 94, 1777.
122. Islam, A. H., Ehara, T., Kato, H., Hayama, M. and Nishizawa, O. 2004, Urology, 64, 468.
123. Martin de las Mulas, J., Reymundo, C., Espinosa de los Monteros, A., Millan, Y. and Ordas, J. 2004, Vet. Comp. Oncol., 2, 24.
124. Mosunjac, M. B., Lewis, M. M., Lawson, D. and Cohen, C. 2000, Diagn. Cytopathol., 23, 151.

125. Hasegawa, M., Moritani, S., Murakumo, Y., Sato, T., Hagiwara, S., Suzuki, C., Mii, S., Jijiwa, M., Enomoto, A., Asai, N., Ichihara, S. and Takahashi, M. 2008, Pathol. Int., 58, 288.
126. Uzquiano, M. C., Prieto, V. G., Nash, J. W., Ivan, D. S., Gong, Y., Lazar, A. J. and Diwan, A. H. 2008, Mod. Pathol., 21, 540.
127. Meehan, K. L., Holland, J. W. and Dawkins, H. J. 2002, Prostate, 50, 54.
128. Tuxhorn, J. A., Ayala, G. E., Smith, M. J., Smith, V. C., Dang, T. D. and Rowley, D. R. 2002, Clin. Cancer Res., 8, 2912.
129. Verone, A. R., Duncan, K., Godoy, A., Yadav, N., Bakin, A., Koochekpour, S., Jin, J. P. and Heemers, H. V. 2013, Carcinogenesis, 34, 1737.
130. Yamamura, H., Yoshikawa, H., Tatsuta, M., Akedo, H. and Takahashi, K. 1998, Int. J. Cancer, 79, 245.
131. Yoshikawa, H., Taniguchi, S. I., Yamamura, H., Mori, S., Sugimoto, M., Miyado, K., Nakamura, K., Nakao, K., Katsuki, M., Shibata, N. and Takahashi, K. 1998, Genes Cells, 3, 685.
132. Yamamura, H., Hashio, M., Noguchi, M., Sugenoya, Y., Osakada, M., Hirano, N., Sasaki, Y., Yoden, T., Awata, N., Araki, N., Tatsuta, M., Miyatake, S. I. and Takahashi, K. 2001, Cancer Res., 61, 3969.
133. Horiuchi, A., Nikaido, T., Taniguchi, S. and Fujii, S. 1999, J. Natl. Cancer Inst., 91, 790.
134. Takeoka, M., Ehara, T., Sagara, J., Hashimoto, S. and Taniguchi, S. 2002, Eur. J. Cancer, 38, 436.
135. Debald, M., Jin, J. P., Linke, A., Walgenbach, K. J., Rauch, P., Zellmer, A., Fimmers, R., Kuhn, W., Hartmann, G. and Walgenbach-Brunagel, G. 2014, Tumour Biol., in press, 2014 Aug 7. [Epub ahead of print].
136. Masuda, H., Tanaka, K., Takagi, M., Ohgami, K., Sakamaki, T., Shibata, N. and Takahashi, K. 1996, J. Biochem., 120, 415.