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Stellate Cell Contraction: Role, Regulation, and Potential Therapeutic Target

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Synopsis

The contraction of hepatic stellate cells has been proposed to mediate fibrosis by regulating sinusoidal blood flow and extracellular matrix remodeling. Abundant data from diverse, yet complementary, experimental methods support a robust model for the regulation of contractile force generation by stellate cells. In this model, soluble factors associated with liver injury, including endothelin-1 and nitric oxide, are transduced primarily through rho signaling pathways that promote the myosin II-powered generation of contractile force by stellate cells. The enhanced knowledge of the role and differential regulation of stellate cell contraction may facilitate the discovery of new and targeted strategies for the prevention and treatment of hepatic fibrosis.

Keywords

hepatic stellate cell; contraction; fibrosis; pericyte; rho-associated kinase; sinusoid

Introduction

Contractile force generation by hepatic stellate cells is recognized to play a key role in the liver's response to injury. This cellular behavior is consequently believed to contribute to normal healing as well as the development of hepatic fibrosis. Therefore, improved understanding of stellate cell contraction and its regulation would be predicted to facilitate development of clinical strategies for the treatment of liver disease. Despite more than 15 years of study, however, effective therapies based on targeting the generation of contractile force by stellate cells have remained elusive. This chapter will examine the current state of knowledge regarding stellate cell contraction, its role, its regulation, and its potential as a therapeutic target, by addressing three fundamental questions:

1. What do we believe we know?

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2. What do we really know?
3. What would we like to know?

What do we believe we know about hepatic stellate cell contraction?

A number of observations in the human liver have led to the concept that stellate cells are contractile and that generation of contractile force by these cells mediates hepatic pathophysiology. Stellate cells express α-smooth muscle actin, a marker of non-muscle cell contractility, in patients with various forms of chronic liver injury [1-6]. Moreover, stellate cells of normal human livers do not express α-smooth muscle actin, suggesting that contractility may be induced by liver injury. Human stellate cells express various receptors for well-known contractile agonists. Studies have demonstrated stellate cell expression of receptors for endothelin-1, arginine-vasopressin, and angiotensin II, all of which induce generation of contractile force by contractile cell types [7-10]. The presence of these receptors suggests that stellate cells are capable of transducing chemical signals into changes in mechanical force. These observations indicate that stellate cells contain the cellular machinery necessary for generation of contractile force.

The anatomic location of stellate cells in the normal human liver also suggests an important role for contraction by these cells in the regulation of sinusoidal blood flow. Immunohistochemical studies of normal human liver show that stellate cells reside in the perisinusoidal space and extend elongate protrusions that run along and encircle one or more sinusoids [3,4,11]. This anatomy is similar to that of tissue pericytes, such as the mesangial cells of the kidney, which modulate vascular tone by contracting around their capillaries [12-14]. In the same way, hepatic stellate cells have been theorized to regulate sinusoidal resistance, and consequently blood flow, by contracting around sinusoids.

Studies of the injured human liver have demonstrated that stellate cells appear prominently in fibrotic bands of collagen (scar tissue) remote from their normal location [1-5,11,15]. This finding raised the possibility that stellate cells may function similar to cutaneous myofibroblasts that participate in wound healing of the skin by contracting scar tissue as healing and regeneration progresses [16-18]. Hence, it has been proposed that contractile force generation by stellate cells may permit remodeling of extracellular matrix during the liver's injury response [19].

In summary, conventional wisdom holds that hepatic stellate cells are contractile, and their capacity to generate contractile force mediates the liver's injury response through modulation of sinusoidal blood flow and scar contracture (Fig. 1). This current understanding of the role of stellate cell contraction is, however, largely based on circumstantial evidence and common logic. In the following section we will dissect the scientific methods that have been employed to study stellate cell contraction, as well as discuss the model (Fig. 2) for the regulation of stellate cell contraction that is best supported by the evidence.

What do we really know about hepatic stellate cell contraction?

Current understanding of hepatic stellate cell contraction has been advanced largely through use of *in vitro* experimental methods. Several methods have been employed to study stellate cell contraction, each with its own inherent limitations in spatial and temporal resolution, accuracy and precision, and fidelity to what occurs *in vivo*. Hence, to properly appreciate what we really know about stellate cell contraction it is worthwhile to appraise the strengths and limitations of the main assays used to study this important stellate cell behavior.

An early technique for the study of stellate cell contraction examined the decrease in two-dimensional cell surface area of cultured cells as a surrogate marker for generation of contractile

force [20-23]. In this assay stellate cells in culture were grown to sub-confluence on a glass cover slip and visualized with transmission light video-microscopy. Reductions in stellate cell surface area could be measured in response to exposure to various agonists and inhibitors. Although this assay permitted quantitative measurements with subcellular spatial resolution and temporal resolution in seconds, changes in stellate cell surface area were not a direct gauge of contractile force. Indeed, shrinkage of stellate cell surface area could result from a number of non-contractile events, including changes in cellular adhesion or volume, three-dimensional shape changes, or even focusing artifacts, making it difficult to discern how accurate a measure of the contractile force generated by stellate cells this method provided.

Hepatic stellate cell contraction has also been evaluated using a model in which stellate cells in culture are grown in a monolayer on silicone rubber-coated coverslips [24-27]. In this assay the silicone rubber substrate, upon which stellate cells were grown, was visualized with transmission light microscopy and wrinkling of the substrate was examined. This method permitted semi-quantitative determination of substrate wrinkling, as a surrogate measure of the tension developed by the population of stellate cells across the silicone rubber, within seconds of exposure to various agonists and inhibitors. Increases in substrate wrinkling likely reflect gross changes in stellate cell contractile force generation, but the tightness of that correlation was unknown. Although determination of substrate wrinkling was subjective and imprecise, this assay did permit examination of the formation and loss of wrinkles, putative correlates of contractile force generation and relaxation, respectively.

Another method for examining stellate cell contraction employed a model in which stellate cells in culture were grown on top of or within gel lattices composed of type-1 collagen [27-35]. In this assay shrinkage of the collagen gels by the population of stellate cells was employed as a surrogate measure of cellular contraction. After release of the gel from a supporting culture dish and exposure to different agonists and inhibitors changes in gel diameter could be measured with a ruler after at least an over night incubation. This technique did not permit measurement of relaxation nor acute changes in contractile force development. In addition, reductions in gel area were not reversible. It is uncertain how closely changes in lattice area correlate with alterations in contractile force generation, and this model may not differentiate between active contraction and passive changes in stellate cell tension (e.g., passive tension across the lattice generated by cellular spreading or increases in stellate cell number).

High resolution intravital videomicroscopy of sinusoids within the isolated rat liver has been used as a model for the study of stellate cell contraction [36-39]. In this assay changes in sinusoidal diameter were visualized adjacent to stellate cell bodies demarcated by retinoid autofluorescence as a surrogate measure of contractile force generation by stellate cells encircling these vessels. These sinusoidal changes could be observed in real time within minutes of exposure to different agonists and inhibitors. By finely measuring and controlling input and output pressures across the major hepatic vessels and estimating sinusoidal blood flow, estimates of increases and decreases in sinusoidal resistance could be derived. This technique, however, did not permit direct measurement of contractile force generation by stellate cells, nor did it rule out the possibility that other cell types (e.g., co-localized endothelial cells or upstream or downstream vascular smooth muscle) or noncontractile events (e.g., cellular swelling) might contribute to the observed changes in sinusoidal diameter.

The contraction of hepatic stellate cells in culture has been directly and quantitatively measured [40-42]. In this assay stellate cells were grown within a three-dimensional type-1 collagen gel lattice, which was placed in an organ bath and attached to a sensitive force transducer. This model permitted real time measurement in actual force units of the contractile tension generated by stellate cells within the gel in response to various agonists and inhibitors. The method

exhibited temporal resolution in the range of seconds, and it permitted precise quantification of both contraction and relaxation within the same sample. Although this technique allowed direct measurement of the contractile forces exerted by stellate cells populating a collagen gel, determination of the contractile force generated by a single stellate cell could only be estimated.

When evaluating the methods employed to study hepatic stellate cell contraction, a critical consideration is how closely the assay is likely to reflect what actually occurs within the human liver, in other words the fidelity of the experimental model. Despite the diverse experimental models and substantial efforts to better understand stellate cell contraction important factors limit what we really know about stellate cell contraction *in vivo*. Except for intravital microscopy, all of the methods used to study stellate cell contraction employed cells in culture. This raises the concern that isolated stellate cells do not function in an identical fashion to stellate cells in the liver. This is particularly true in studies that employed secondary cultures of stellate cells [7,33,41,43]. Thus, it is generally recognized that the results of experiments performed using stellate cells in primary culture may have greater clinical relevance. Another point is that experiments performed on stellate cells in monolayer do not replicate the normal three-dimensional environment in which stellate cells reside *in vivo*. Therefore, studies of stellate cells grown within collagen gels more likely replicate the authentic milieu within the liver. However, intravital microscopy of hepatic sinusoids in intact liver, both *in vivo* and *ex vivo* [36-39], are most likely to be relevant to human pathophysiology. Despite the shared and unique limitations of each of the different methods used to study stellate cell contraction, together these assays complement each other and have contributed to a robust understanding of stellate cell contraction.

A number of chemicals have been demonstrated to stimulate stellate cell contraction, including endothelin-1, arginine-vasopressin, angiotensin-II, thrombin, eicosanoids, and α1-adrenergic agonists [9,10,20,24,35,40-42]. The best-studied and most prominent agonist for stellate cell contraction is endothelin-1. Circulating levels of this peptide are elevated in patients with liver disease [7,44,45], and increased in animal models of liver injury [46,47]. Endothelin-1 can induce markers of stellate cell contraction in every one of the assays discussed earlier [20,25, 29,36,40]. In particular, the magnitude and speed of the contractile force generated by stellate cells in response to endothelin-1 has been predicted to be sufficient to regulate sinusoidal resistance to blood flow [40]. Even more significant, perfusion of isolated rodent livers with endothelin-1 caused a reduction in sinusoidal diameter colocalized with stellate cells that was paralleled by an increase in portal pressure [36,48-51]. Moreover, administration of endothelin-1 receptor antagonists decreased portal pressure in portal hypertensive rats [52]. These experimental findings indicate that endothelin-1 is a potent agonist of stellate cell contraction and suggest an important contribution of this mediator to the regulation of hepatic blood flow.

Several agents, including nitric oxide, carbon monoxide, and prostaglandins, may counteract the effects of contraction-inducing stimuli by causing stellate cell relaxation [24,25,38, 53-55]. Nitric oxide production is reduced in the injured liver [56-58]. *In vitro* studies have suggested that activation of nitric oxide signaling (through nitric oxide donors or cytokine stimulation of nitric oxide production) causes relaxation in stellate cells and attenuates agonist-induced contraction [10,25,53,56,59,60], a process that might occur through cGMP-dependent activation of myosin light chain phosphatase, similar to what has been demonstrated in smooth muscle cells [61-63]. Finally, nitric oxide donors can attenuate elevations in portal pressure in the perfused rodent liver induced by endothelin-1 or other contraction-inducing stimuli [36, 48,64]. These observations have led to a proposed model in which sinusoidal tone is finely modulated by the net balance of agents that induce stellate cell relaxation, such as nitric oxide, and agonists of stellate cell contraction, such as endothelin-1 [65-67].

It has long been known that the motor protein complex, myosin II, powers contractile force generation in smooth muscle and fibroblasts through its action on the actin cytoskeleton [68, 69]. Numerous studies observed that hepatic stellate cells in culture express both myosin II [31,41,42,70-73] and a fully formed actin cytoskeleton [31,41-43,70-74]. Myosin II activation, as assessed by myosin regulatory light chain phosphorylation, correlates with various surrogate measures of stellate cell contraction [31,43,71], as well as with the actual contractile force generated by stellate cells [41]. Moreover, antagonism of myosin phosphorylation inhibited contractile force generation by stellate cells [42]. Finally, the myosin regulatory light chain expressed by stellate cells is phosphorylated at serine 19 [73], the consensus activation site for myosin II. Taken together these results indicate that stellate cell contraction is powered by myosin II, which is activated by phosphorylation of its myosin regulatory light chain.

Evidence suggests that Ca^{2+} signaling pathways regulate stellate cell contraction by activating myosin light chain kinase, which selectively phosphorylates the myosin regulatory light chain [20,75-77], similar to what has been demonstrated in smooth muscle. This notion was supported by several experimental observations. First, ligands including endothelin-1, thrombin, and angiotensin II, that induced transient increases in cytosolic Ca^{2+} concentration also stimulated stellate cell contraction [7,10,20,25,40,41]. Second, plasma membrane Ca^{2+} channel expression, Ca^{2+} influx through these channels, and cytosolic Ca^{2+} concentration, each correlated with reductions in stellate cell surface area [23,60,77]. Third, inhibitors of Ca^{2+}-dependent myosin light chain kinase attenuated the shrinkage of collagen gels populated with stellate cells [35,43]. Although these findings suggested an important role for Ca^{2+} signaling in the control of stellate cell contraction, they did not provide any direct evidence to support this model.

In contrast to previously held views, current data indicate that Ca^{2+} signaling pathways play a subordinate role in the regulation of contractile force generation by stellate cells. The contribution of Ca^{2+} signaling pathways to the regulation of stellate cell contraction was directly tested by modulating cytosolic Ca^{2+} and directly measuring the contractile force generated by this cell type [42]. Increases in cytosolic Ca^{2+} induced by depolarizing the plasma membrane did not provoke contractile force generation. Superphysiological elevations in cytosolic Ca^{2+} triggered by a calcium ionophore induced minimal increases in contractile force. Eliminating increases in cytosolic Ca^{2+} with a calcium chelator had no effect on endothelin-1-induced contractile force generation. This study provided surprising evidence indicating that Ca^{2+} signaling is neither necessary for contractile force generation by stellate cells, nor is it sufficient to provoke stellate cell contraction. This fresh perspective was supported by the recent observation stellate cell contraction was stimulated by the inhibition of myosin phosphatase despite the absence of any changes in cytosolic Ca^{2+} concentration [35].

Over the past decade substantial data have emerged demonstrating that contractile force generation by certain non-muscle cell types, including fibroblasts and endothelial cells, is predominantly regulated by transduction pathways that signal through the ras-like GTPase, rhoA, rather than Ca^{2+} [69,78-80]. Mounting evidence indicate that rho signaling pathways also control stellate cell contraction. Stellate cells express rhoA and rho-associated kinase [31,70,71]. Specific inhibition of rhoA caused derangement of the stellate cell actin cytoskeleton [70,74]. Highly selective antagonism of the rhoA effector protein, rho-associated kinase, impeded shrinkage of collagen gels populated with stellate cells [31,43,71], inhibited myosin regulatory light chain phosphorylation [31,41-43,71,73], and blocked contractile force generation by stellate cells [41,42]. Attenuation of myosin phosphatase also reduced stellate cell contraction as assessed by the shrinkage of stellate cell-populated collagen gels [35]. In combination with studies of the contribution of Ca^{2+} signaling, these studies support a model in which contractile force generation by stellate cells is mediated primarily by rho signal transduction pathways.

With regards to the putative roles that stellate cell contraction may play in the pathophysiology of the liver, the strongest data pertains to their contribution to the modulation of sinusoidal blood flow. The concept that stellate cells modulate resistance to hepatic blood flow by contracting around sinusoids is supported by several observations. First, stellate cells *in situ* exhibit a pericyte-like morphology with protrusions encircling the sinusoids [81-83]. Second, the number and spacing of stellate cells and their characteristic protrusions overlay the entire sinusoidal network [84]. Third, *ex vivo* perfusion of the liver with endothelin-1 induced reductions in sinusoidal caliber colocalized with stellate cells [36,37,85]. Fourth, direct measurement of contractile force generation by stellate cells within collagen gels suggests that the magnitude and rate of stellate cell contraction and relaxation are capable of modulating blood flow via sinusoidal constriction [40]. Taken together these findings obtained from several complementary methods indicate that stellate cells contribute to the regulation of sinusoidal blood flow.

What would we like to know about hepatic stellate cell contraction?

What we would really like to know about how the emerging pathobiology of stellate cell contraction can be used to develop new strategies for the prevention and treatment of hepatic fibrosis in humans. Despite fifteen years of intensive investigation and a great deal of new information about the role and regulation of stellate cell contraction, no effective stellate cell contraction-targeted therapies for hepatic fibrosis have been validated. In fact, no fibrosis directed treatments of any sort have yet been developed for the treatment of chronic liver disease [86-89]. As discussed in other chapters of this monograph, the only proven therapies for fibrosis so far are directed at the prevention or removal of a specific cause of chronic hepatic injury, such as treatment of hepatitis C or biliary obstruction.

Two logical approaches for developing new treatments for hepatic fibrosis are (1) to destroy stellate cells or disable their function and (2) to modulate specific molecular targets within key signal transduction pathways used by stellate cells. There are, however, serious real and theoretical challenges to these general therapeutic approaches. Stellate cells mediate the response of the liver to both acute and chronic injury. Hence, they are believed to contribute to both the normal wound healing process, as well as to the development of hepatic fibrosis and subsequent cirrhosis. If this is accurate, then destruction or disabling of stellate cells could impair healthy and essential responses of the liver to injury in addition to the anticipated prevention or attenuation of fibrosis. One solution to the paradox that the presence of intact stellate cells may be necessary for both normal wound healing and fibrogenesis might be to selectively target influential signaling pathways used by stellate cells. The problem with this therapeutic strategy is that important signaling pathways are generally shared by amongst different cell types. For example, employment of a mitogen-activated protein kinase antagonist to inhibit stellate cell proliferation would also be predicted to influence the proliferation of hepatocytes, and numerous other cell types. Therefore, it would be especially advantageous to identify and develop treatment strategies precisely and specifically targeted to stellate cell behaviors that mediate hepatic fibrosis.

The contractile force exerted by stellate cells contributes to the regulation of sinusoidal blood flow and the development of fibrosis. As discussed, emerging evidence indicates that generation of contractile force by stellate cells may be differentially regulated by transduction pathways that signal through rho and rho-associated kinase, rather than Ca^{2+} and myosin light chain kinase as it is in vascular smooth muscle. This differential regulation of stellate cell contraction offers the possibility that novel therapeutic strategies could be developed that selectively target the generation of contractile force by stellate cells. In fact, commercially available highly selective small molecule inhibitors of rho-associated kinase attenuate the increases in intrahepatic vascular resistance and portal hypertension [35,71,90,91] and lessen

the development of hepatic fibrosis [91-94] in diverse rodent models of liver injury. These studies provide proof-of-principle that stellate cell contraction can be selectively targeted to treat hepatic fibrosis, at least in rodent models of chronic liver injury.

A significant concern with targeting rho signal transduction pathways as a strategy for treating hepatic fibrosis is that this pathway is ubiquitous in playing vital roles in diverse cell types throughout the body. This concern could be circumvented by delivering inhibitors directly to the liver or stellate cells. Possible methods for directed delivery include portal or hepatic venous injection, coupling drugs to carriers (e.g., antibodies, peptides, lectins, or lipids) with affinity for the liver or stellate cells, and the use of particular viruses to selectively deliver therapeutic genes or ribonucleic acids [71,95-99] to stellate cells or the liver. By integrating new technologies for liver-directed delivery with pharmaceutical or genetic agents that selectively target stellate cell contraction it may be possible to develop effective strategies for the prevention and treatment of hepatic fibrosis.

Summary

The contractile force generated by stellate cells within the liver may contribute to the development of hepatic fibrosis by modulating sinusoidal blood flow and participating in extracellular matrix remodeling. For more than fifteen years, the role and regulation of stellate cell contraction have been areas of substantial research. Diverse, but complementary, experimental methods have been employed to elucidate the pathophysiology of stellate cell contraction. Although each technique for studying the contraction of stellate cells has its own limitations, taken together the published studies have provided a robust model for the regulation of stellate cell contractile force generation. In this model, soluble factors associated with liver injury, including endothelin-1 and nitric oxide, are transduced primarily through rho signaling pathways that promote the myosin II-powered generation of contractile force by stellate cells. Moreover, compelling data support a role for stellate cells in the control of hepatic blood flow by contracting around sinusoids. Our enhanced understanding of the role and differential regulation of stellate cell contraction may facilitate the discovery of new and targeted strategies for the prevention and treatment of hepatic fibrosis.

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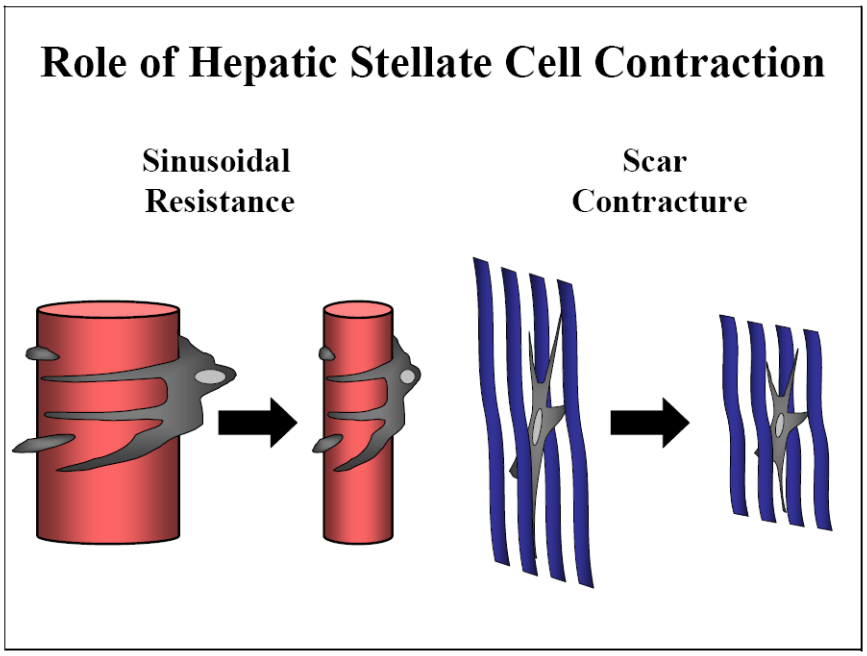

Fig. 1.
Role of hepatic stellate cell contraction. Stellate cell contractile force generation is thought to mediate the liver's response to injury through constricting sinusoids and contracting scar.

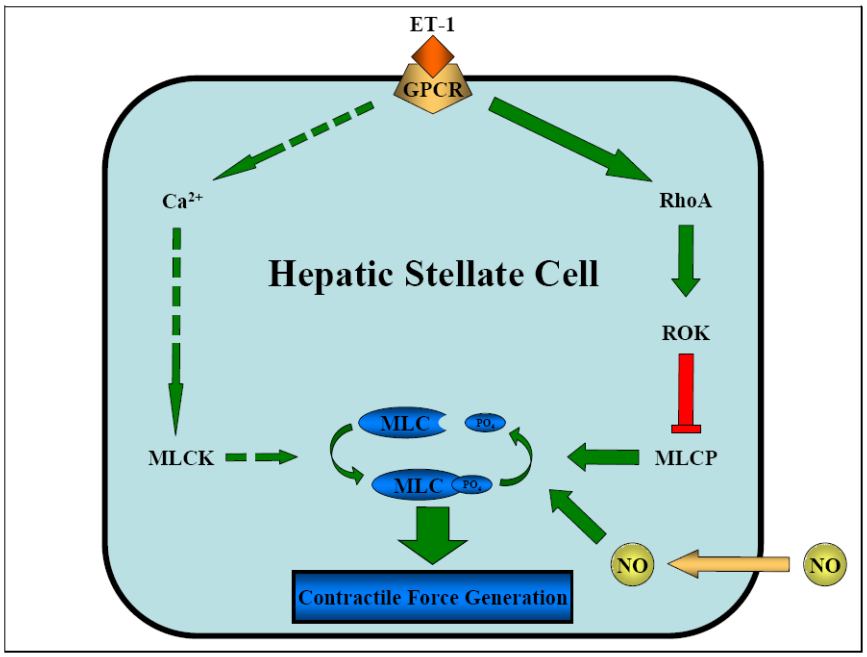

Fig. 2.

Regulation of hepatic stellate cell contraction. Soluble factors associated with liver injury, such as endothelin-1 and nitric oxide, are transduced primarily through rho signaling pathways that promote the myosin II-powered generation of contractile force by stellate cells. Dashed arrows indicate subordinate Ca^{2+} signaling pathway. ET-1, endothelin-1; GPCR, G protein coupled receptor; MLCK, myosin light chain kinase; RhoA, rho family GTPase; ROK, rho-associated kinase; MLCP, myosin light chain phosphatase; NO, nitric oxide; MLC, myosin light chain; PO_4, phosphate.