Review Article

RhoA/ROCK pathway: implication in osteoarthritis and therapeutic targets

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Abstract: Ras homolog gene family, member A (RhoA) and its downstream effector Rho-associated protein kinase (ROCK) play important roles in multiple cellular processes, but abnormal activation of this pathway have been reported to be involved in various types of diseases, including osteoarthritis (OA). This article focused to review the RhoA/ROCK association and its functional role in OA development, and possible therapeutics of OA by targeting this pathway. We have explored the databases like Pubmed, Google Scholar, Web of Science and SCOPUS, and collected the papers on Rho/ROCK and their relationship with OA, and reviewed comprehensively. Studies revealed that the abnormal activation of RhoA/ROCK signaling is involved in early phase response to abnormal mechanical stimuli, which is thought to be a contributory factor to OA progression. RhoA/ROCK interacts with OA pathological factors and induces cartilage degeneration through the degradation of chondrocyte extracellular matrix (ECM). As the RhoA/ROCK activity can affect bone formation by triggering cartilage degradation, it may represent a possible therapeutic target to treat OA. Interestingly, several pharmaceutical companies are investing in the development of RhoA/ROCK inhibitors for the treatment of OA. However, a few in vivo experiments have been successfully conducted to demonstrate the potential value of RhoA/ROCK pathway inhibition in the treatment of OA. This review provides an insight into the functional role of Rho/ROCK pathway, and indicates that targeting this pathway might be promising in future OA treatment.

Keywords: Ras homolog gene family, Rho-associated protein kinase, cartilage degeneration, osteoarthritis, RhoA inhibitor, ROCK inhibitor

Introduction

Ras homolog gene family, member A (RhoA) is a small GTPase protein in the Rho family. In humans, RhoA is encoded by the gene RHOA, is located on chromosome 3 and consists of an effector domain, four exons, a hypervariable region and a CAAX box motif (C: Cys; A: aliphatic residue; X: any residue) [1, 2]. The N-terminus region of RhoA contains two switch regions, Switch I and Switch II that have characteristic folding, corresponds to specific regions on the RhoA coil and is uniformly stabilized via hydrogen bonds. The conformations of these switches are modified following the activation or inactivation of the protein. The C-terminus of RhoA is essential for correct localization of the protein [3, 4]. RhoA protein is expressed in all tissues including normal human tissues, embryonic tissues and stem cells. RhoA localizes predominantly in the plasmatic membrane and cytoplasm, as well as near the cell-cell contacts and cell projections. RhoA plays an important role in multiple cellular processes such as cell growth, transformation, and cytoskeleton regulation, mostly actin stress fibers formation and actomyosin contractility [5-7]. Rho-associated protein kinase (ROCK) is a downstream effector of RhoA, which exists in two isoforms, ROCK1 and ROCK2. Rho/ROCK signaling pathway is an important signal transduction system that is critically involved in cell growth, differentiation,
migration and development [8, 9]. This pathway is also required for neurite outgrowth, bone formation, dorsal closure and myogenesis [10]. Deregulation of this pathway is associated with different kinds of diseases. Abnormal activation of this pathway has been reported to be involved in various types of cancers as well as other diseases including neurological disorders, diabetes, asthma, hypertension and osteoarthritis [11-16].

Osteoarthritis (OA) is a common degenerative joint disorder, predominantly occurring in both sexes as people become older. This condition is progressive and directs to functional decline and loss in quality of life. OA is primarily characterized by destruction of the articular cartilage, presence of subchondral bone lesions, and associated synovitis [17, 18]. Degradation of the articular cartilage is the main cause of the pathogenesis of OA. Maintaining the chondrocytes emerges to be an important factor for preventing the entire cartilage and its degeneration [19, 20]. The major symptoms of OA include joint pain, joint stiffness and reduced motion. It affects 240 million people globally, about 10% of men and 18% of women. The epidemiology of the disorder is complex and several risk factors are relevant to the initiation and development of OA, including aging, obesity, inflammation, trauma, genetics, biological and biomechanical components [21-23]. OA cannot be cured totally. Nowadays, the management of OA focuses on the alleviation of symptoms. International recommendations for the initial management of OA include three main categories: non-pharmacological, pharmacological and surgical [24, 25]. Recent development of OA treatment and management includes cell therapy via intra-articular injection, maintaining homeostasis by regulating renin-angiotensin system, targeted delivery of mesenchymal stem cells (MSCs) for cartilage regenerations, and of course, exercise is a very effective strategy to fight against OA [26-29]. Among pharmacological therapies, extended-release triamcinolone and conventional disease-modifying anti-rheumatic drugs (DMARDs) are also worth mentioning [30]. However, these treatments are not always satisfactory because the conservative treatments are highly expensive and are not powerful enough to inhibit OA progression. Moreover, the conventional imaging techniques can detect only quite advanced disease [21, 31]. To overcome these problems, identification of the sources and mechanisms of pain in OA is important. A better understanding of the pathogenesis of OA may develop new and successful approaches for the treatment of OA, and may also potentially help identify alternative therapies to help reduce symptoms and improve function.

In this study, we have focused on the association of RhoA/ROCK in the development and progression of OA, and evaluated the efforts to target this pathway to ameliorate disease conditions.

RhoA/ROCK interrelation

Members of Rho-associated coiled-coil kinases (ROCKs) were initially discovered as downstream targets of RhoA. ROCK belongs to the family of serine/threonine kinases and includes two isoforms: ROCK1 and ROCK2. ROCK1 is mainly expressed in non-neural tissues, such as lung, kidney and skeletal muscle whereas ROCK2 is mainly expressed in the central nervous system, including hippocampal pyramidal neurons, cerebral cortex and cerebellar Purkinje cells [32-34]. The ROCK proteins are structurally composed of a catalytic kinase domain (N-terminal) followed by α-helical coiled-coil region containing the Rho-binding domain (RBD) and a cysteine-rich domain at the carboxyl-terminal region (C-terminal) containing a pleckstrin homology (PH) domain [35, 36]. Upon interaction of the RBD domain with GTP-loaded RhoA, the association of the C-terminus with the N-terminal kinase domain has been disrupted, leading to the activation of ROCK [37, 38]. The activation of the RhoA/ROCK pathway has been implicated in a wide range of biological processes including stress fiber formation, cytoskeletal reorganization and migration, adhesion, motility, differentiation, maturation, apoptosis and remodeling of the extracellular matrix (ECM), survival, and gene expression as well as reduces chondrocyte and osteogenic differentiations [39-41]. Following activation of RhoA, ROCK proteins serve as major downstream effectors for mediating actin depolymerization and actomyosin contraction (Figure 1). ROCK protein phosphorylates myosin II regulatory light chains (MRLC) and promotes the phosphorylation of myosin light chain (MLC) through phosphorylation and inactivation of
RhoA/ROCK pathway in osteoarthritis

Alteration in cytoskeletal remodeling and hypertrophy of articular chondrocytes has been implicated in the pathogenesis of OA. Chondrocyte hypertrophy is one of the characteristics of OA. During hypertrophy, collagenase activity and cellular and matrix changes are known to be induced by metabolically active hypertrophic chondrocytes [44, 45]. RhoA/ROCK signaling pathway is recognized as a critical regulator of tissue response to injury as this has the ability to modulate a wide range of biological processes. However, dysregulated RhoA/ROCK activity has been implicated in several human pathophysiological conditions [46, 47]. RhoA/ROCK signaling is involved in early phase response to abnormal mechanical stimuli through the modification of the chondrocyte actin cytoskeleton and imbalance of β-catenin, which is thought to be a contributory factor to OA progression. RhoA/ROCK affect the OA pathological factors such as transforming growth factor (TGF), epidermal growth factor receptor (EGFR), interleukin-1 (IL-1), insulin-like growth factor-1 (IGF-1) and leptin (Table 1) and induces the degradation of chondrocyte ECM. Then the chondrocytes lose their stable phenotype and undergo hypertrophy-like changes with the expression of various chondrocyte maturation and osteogenesis markers such as COLX, MMP-13, a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-5, Runx2 and vascular endothelial growth factor (VEGF). The hypertrophy-like changes play a critical part in the development of OA by protease mediated cartilage degradation [13, 48, 49] (Figure 2). Wang et al. showed that both RhoA and ROCK1/2 are expressed throughout chondrogenic differentiation of primary cells and ATDC5 (chondrogenic cell line derived from mouse teratocarcinoma) cells. They found that protein levels of RhoA and ROCK1 increase at late (hypertrophic) stages of ATDC5 differentiation while this effect has not seen at the RNA level due to the posttranscriptional regulation of protein levels [50]. IL-1, a pro-inflammatory cytokine highly expressed in OA, has been found to modulate F-actin remodeling in chondrocytes [48, 51]. Liang et al. suggested that

**Figure 1.** RhoA/ROCK signaling. GTP-loaded RhoA interacts with RBD domain of ROCK activity and induces the activation of RhoA/ROCK signaling. The activation of the RhoA/ROCK pathway has been implicated in a wide range of biological processes. Following activation of RhoA, ROCK proteins serve as major downstream effectors for mediating actin depolymerization and actomyosin contraction. Substrates of ROCK include MLC, MLCP and LIMK. Phosphorylation of the substrates of ROCK such as MLC and LIMK is involved in the stabilization of actin filaments and actomyosion contraction [2, 38, 43].

**MLC phosphatase or direct phosphorylation of MLC, leading to the activation of myosin II and enhancement of the actomyosin-driven contractility. In addition, there is ROCK-mediated phosphorylation and concomitant inactivation of the myosin-binding subunit (MYPT1) of MLC phosphatase (MLCP). ROCK also phosphorylates LIM motif containing protein kinase (LIMK), which in turn phosphorylates the F actin-severing protein cofilin and inactivates its actin depolymerization activity, leading to stabilization of actin filaments [2, 42, 43].**

RhoA/ROCK in osteoarthritis

Alteration in cytoskeletal remodeling and hypertrophy of articular chondrocytes has been implicated in the pathogenesis of OA. Chondrocyte hypertrophy is one of the characteristics of OA. During hypertrophy, collagenase activity and cellular and matrix changes are
Table 1. Effects of RhoA/ROCK signaling on pathological factors in the progression of OA

<table>
<thead>
<tr>
<th>Contributors</th>
<th>Mechanism</th>
<th>Consequences</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>β-catenin</td>
<td>OA-like cartilage degradation, osteophyte formation, and MMP13 expression</td>
<td>Induces chondrocyte hypertrophy and maturation</td>
<td>[13]</td>
</tr>
<tr>
<td>Wnt/β-catenin</td>
<td>Up-regulation of Wnt16/WISP-1, down-regulation of FRZB, upregulation of β-catenin, axin-2, C-JUN and LEF-1</td>
<td>Induces articular chondrocyte catabolism, hypertrophy-like changes and cartilage degradation</td>
<td>[13]</td>
</tr>
<tr>
<td>IL-1</td>
<td>Inhibits osmotically induced calcium signaling and volume regulation in articular chondrocytes</td>
<td>Modulates F-actin remodeling in chondrocytes</td>
<td>[49]</td>
</tr>
<tr>
<td>Leptin</td>
<td>A activates the RhoA/ROCK/LIMK/cofilin pathway</td>
<td>Induces cytoskeletal reorganization in chondrocytes, accompanied by change of cell shape and stress fiber formation</td>
<td>[50]</td>
</tr>
<tr>
<td>TGF-α</td>
<td>Excess type II collagen and aggrecan degradation</td>
<td>Induces articular cartilage degradation</td>
<td>[53]</td>
</tr>
<tr>
<td>EGF</td>
<td>Increases MMP-13 production</td>
<td>Promotes cartilage matrix degradation</td>
<td>[54]</td>
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</table>

There is a direct linkage between cytoskeletal remodeling in chondrocytes and the disease development of OA. It has been reported that the modifications to chondrocyte shape may delay the normal functions of the chondrocyte. They reported the possible involvement of leptin and the RhoA/ROCK pathway in the pathogenesis of OA. Their study demonstrated that leptin activates the RhoA/ROCK pathway and induces cytoskeletal reorganization in chondrocytes, accompanied by change of cell shape (e.g., flattening, elongation, and hypertrophy) and stress fiber formation [13]. Transferring growth factor-α (TGF-α), an activator of epidermal growth factor receptor (EGFR) signaling is upregulated by articular chondrocytes in OA. Stimulation of EGFR signaling in articular chondrocytes by TGF-α resulted in the activation of RhoA/ROCK pathway which induces articular cartilage degradation in OA [49]. Wnt/β-catenin signaling activation has been reported to be involved in articular chondrocyte catabolism, hypertrophy-like changes and cartilage degradation which are the hallmarks of OA progression. RhoA/ROCK signaling can function as a key mediator of β-catenin nuclear translocation in OA progression by inducing chondrocyte hypertrophy and maturation [48, 52]. Epidermal growth factor (EGF) activates Rho family of small GTPases in chondrocyte and promotes MMP-13 production which results in pathological changes in articular cartilage similar to those observed in human OA [13].

RhoA/ROCK in osteoarthritis therapeutics

Identification and characterization of therapeutic targets for OA is exceedingly important for addressing the increasing burden of disease. With increasing recognition of the role of RhoA/ROCK on OA, their activities have been
investigated using both human genetic studies and animal models [49, 53]. RhoA/ROCK activity can affect bone formation by triggering cartilage degradation and therefore may represent a possible therapeutic target to treat OA. RhoA/ROCK signaling is thought to be involved in OA initiation and progression by responding to abnormal mechanical stimuli. An altered level of RhoA/ROCK in articular chondrocytes might be recognized as a new marker for the development of OA [52, 54-56].

Several pharmaceutical companies are investing in the development of RhoA/ROCK inhibitors for the treatment of OA. A few number of small molecule inhibitors of RhoA have been developed and reported in the basic science research [40, 48, 57] (Table 2). It is reported that RhoA inhibitor, AS1892802 displayed alleviation of cartilage damage in rodent OA models. Takeshita et al. injected AS1892802 into the ipsilateral knee or administered p.o. for 3 weeks and found that the compound significantly inhibited cartilage damage. They also found that in vitro, the compound could induce chondrocyte differentiation in a chondrogenic cell line [58]. ROCK inhibitors, such as fasudil (composed of the isoquinoline ring and the pendant ring of the seven-membered homopiperazine) and Y-27632 (can inhibit phenylephrine induced contraction of a rabbit aortic strip and contains a 4-aminoypyridine ring) have been utilized for inflammatory disorders that can function in an ATP-competitive manner [40, 59, 60]. In a study, Wang et al. [50] used Y27632 to block ROCK activity in wild type ATDC5 cells. They showed that ROCK inhibition causes a reproducible increase in alkaline phosphatase and mineralization activity. They examined that treatment of ATDC5 cells with Y27632 partially rescues the effects of RhoA overexpression, inhibits chondrocyte proliferation and accelerates hypertrophic differentiation. Appleton et al. reported that stress fiber formation and contractility may represent unfavorable modification of the articular chondrocyte phenotype through activation of RhoA/ROCK signaling. They showed that RhoA/ROCK signaling suppresses chondrogenesis through the control of Sox9 expression and actin organization. In their study, they suggested that inhibition of RhoA/ROCK signaling effectively reduces articular cartilage degeneration, favors articular cartilage maintenance and repair [49]. Both inhibition of actin polymerization by cytochalasin D and stabilization of existing actin filaments by jasplakinolide resulted in increased Sox9 mRNA level that is important in maintaining the chondrocyte phenotype [51, 61]. Rho GTPases are known to control connective tissue growth factor (CTGF/CCN2, a matrix associated protein from the CCN family) which is suspected to play critical roles in cartilage repair [62]. Nishida et al. reported that a single injection of recombinant CTGF/CCN2 (rCTGF/CCN2) incorporated in gelatin hydrogel (rCTGF/CCN2-hydrogel) into the joint cavity of MIA-induced OA model rats possessed the ability to repair damaged articular cartilage [63]. Targeting RhoA in the treatment of OA has been used only for the last few years. Based on the current scientific studies, the strategy of targeting RhoA for OA prognosis and therapy is instructive for clinical treatment of OA [48]. To improve the therapeutic efficacy of regenerated cartilage, the defined role of RhoA in OA progression should be fur-

### Table 2. The use of therapeutic agents that target RhoA/ROCK in the treatment of OA

<table>
<thead>
<tr>
<th>Therapeutic agents</th>
<th>Experimental models</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS1892802</td>
<td>Monoiodoacetate-injected rats</td>
<td>Inhibited cartilage damage through the inhibition of IL-1β or bradykinin-induced prostaglandin E(2) production</td>
<td>[57]</td>
</tr>
<tr>
<td>Y27632</td>
<td>ATDC5 cell line</td>
<td>Increased alkaline phosphatase and mineralization activity, inhibited chondrocyte proliferation and accelerated hypertrophic differentiation</td>
<td>[48]</td>
</tr>
<tr>
<td></td>
<td>Rat chondrocytes and osteochondral explants</td>
<td>Prevented cytoskeletal rearrangements and stress fiber formation</td>
<td>[53]</td>
</tr>
<tr>
<td></td>
<td>ATDC5 cell line</td>
<td>Increased glycosaminoglycan synthesis and elevated expression of the chondrogenic transcription factor Sox9</td>
<td>[60]</td>
</tr>
<tr>
<td>Cytochalasin D</td>
<td>ATDC5 cell line</td>
<td>Maintained the chondrocyte phenotype through the inhibition of actin polymerization and increased Sox9 level</td>
<td>[60]</td>
</tr>
<tr>
<td>Jasplakinolide</td>
<td>ATDC5 cell line</td>
<td>Maintained the chondrocyte phenotype through the stabilization of actin filaments and increased Sox9 level</td>
<td>[60]</td>
</tr>
<tr>
<td>rCTGF/CCN2</td>
<td>Monoiodoacetate-injected rats</td>
<td>Regenerated the articular cartilage</td>
<td>[63]</td>
</tr>
</tbody>
</table>
ther investigated with animal models utilizing both genetic and pharmacological tools.

Future research

The RhoA/ROCK pathway is a central coordinator of tissue injury response. Dysregulation of this pathway may play potential pathophysiological roles in the development of OA. Therefore, RhoA/ROCK signaling might be good targeting candidates to develop small molecule drugs for OA therapy [41, 56]. Although blocking the activity of RhoA/ROCK is able to prevent chondrocytes from undergoing hypertrophy and ossification, there are several relevant problems to be solved before this strategy can be utilized in OA therapy. Moreover, this pathway is important for many cellular processes and thus targeting RhoA/ROCK could also have unwanted consequences. The dosage and timing of intervention and drugs targeting specific effectors should be carefully investigated to avoid side effects [53]. There is a high degree of homology between the kinase domains and it is difficult to attribute specific functions to either of the two ROCK isoforms. Hence, there is a need for more selective ROCK inhibitors to allow testing in vitro and in clinical studies for the treatment of OA [33, 38, 64].

Conclusion

OA is a chronic and debilitating disease in human that frequently results in progressive degradation of articular cartilage. Currently, there is no effective drug and agent for treating OA. Early diagnosis and administration of effective treatment may be the best strategies against OA. Proper investigation of the modulating factors that might control the symptoms may improve the potential therapy for OA. RhoA/ROCK pathway plays an important role in the development of OA and that inhibition of their activities by selective inhibitors would be beneficial in treating OA. However, a few successful in vivo experiments have been conducted to demonstrate the potential value of RhoA/ROCK pathway inhibition in the treatment of OA. More investigations are necessary to determine the biology of RhoA/ROCK signaling, to develop the strategy of targeting RhoA/ROCK for OA prognosis and to establish novel therapeutics to treat OA in the future.

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Disclosure of conflict of interest

None.

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RhoA/ROCK pathway in osteoarthritis


