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Signaling Through Rho GTPase Pathway as Viable Drug Target

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Abstract

Signaling through the Rho family of small GTPases has been increasingly investigated for their involvement in a wide variety of diseases such as cardiovascular, pulmonary, and neurological disorders as well as cancer. Rho GTPases are a subfamily of the Ras superfamily proteins which play essential roles in a number of biological processes, especially in the regulation of cell shape change, cytokinesis, cell adhesion, and cell migration. Many of these processes demonstrate a common theme: the rapid and dynamic reorganization of actin cytoskeleton of which Rho signaling has now emerged as a major switch control. The involvement of dynamic changes of Rho GTPases in disease states underscores the need to produce effective inhibitors for their therapeutic applications. Fasudil and Y-27632, with many newer additions, are two classes of widely used chemical compounds that inhibit Rho kinase (ROCK), an important downstream effector of RhoA subfamily GTPases. These inhibitors have been successful in many preclinical studies, indicating the potential benefit of clinical Rho pathway inhibition. On the other hand, except for Rac1 inhibitor NSC23766, there are few effective inhibitors directly targeting Rho GTPases, likely due to the lack of optimal structural information on individual Rho-RhoGEF, Rho-RhoGAP, or Rho-RhoGDI interaction to achieve specificity. Recently, LM11A-31 and other derivatives of peptide mimetic ligands for p75 neurotrophin receptor (p75^{NTR}) show promising effects upstream of Rho GTPase signaling in neuronal regeneration. CCG-1423, a chemical compound showing profiles of inhibiting downstream of RhoA, is a further attempt for the development of novel pharmacological tools to disrupt Rho signaling pathway in cancer. Because of a rapidly growing number of studies deciphering the role of the Rho proteins in many diseases, specific and potent pharmaceutical modulators of various steps of Rho GTPase signaling pathway are critically needed to target for therapeutic intervention in cardiovascular disease, neurological disorders, and cancer progression.

Keywords

Rho GTPase; ROCK; Y-27632; fasudil; LM11A-31; NSC23766; CCG-1423

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INTRODUCTION

Rho GTPases represent a subset of the larger Ras superfamily and consist of over 20 intracellular signaling proteins. They are often referred to as the small GTPases because of their ~20kD size, with the most thoroughly characterized members being RhoA, Cdc42, and Rac1 [1]. They are ubiquitously expressed proteins known to be molecular switches for the transduction of signals from external stimuli through the activation of integrins, growth factor receptors, ion channels, and G-protein coupled receptors (Fig 1A). The activation of the GTPase by receptor or non-receptor dependent mechanisms mediates the transition between an active GTP bound state and an inactive GDP bound state [2, 3]. Numerous studies have demonstrated the important roles of Rho GTPases in the regulation of gene transcription, cell proliferation, migration, cell division, and cell shape change [4-6].

The regulation of Rho GTPase activity is controlled by a group of regulatory proteins that are specific for each family member (Fig 1B). These regulatory proteins fall into three general categories: Guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDIs). GEFs act to enhance the exchange of GDP for GTP and thus activate GTPases. GAPs control the hydrolysis of GTP to GDP and are integral in maintaining an appropriate basal level of activity. GDI proteins have varying affinities for different GTPases and keep them from being activated. The differential regulation by the GEFs, GAPs and GDIs allows for selective spatial activation/inactivation of Rho proteins in the cells [7].

The Rho proteins have over 60 known downstream effectors, which determine the outcome of the activation for a given Rho GTPase protein. The downstream target of the Rho GTPases dictates if the activation plays a role in cell morphology, polarity, vesicular trafficking, or cell cycle control [4, 8]. After the discovery of the Rho GTPases, the roles of the individual proteins are determined from experiments using a constitutively active form of the protein. The active form of the protein is expressed in cultured fibroblast cells and is shown to have major effects on cell morphology [9]. Activated RhoA influences the tension of cell attachment to the substrate, which is supported by the actin abundant stress fiber in the cell. Rac1 and Cdc 42, on the other hand, affect the formation of peripheral projections rich in actin like lamellipodia and filopodia. It is originally thought that these proteins are active only in fibroblasts but it is now clear that the morphology of many eukaryotic cells, such as neurons, can be altered by activation of Rho GTPases [6]. The most common action of Rho GTPase activation on mammalian cells is through the reorganization of actin cytoskeleton.

A number of Rho downstream targets have been identified, including PKN which is protein kinase C (PKC)-like kinase [10], Rhotekin [11], Citron [12], PIP5-kinase (phosphatidylinositol 4-phosphate 5-kinase)[13], and p140mDia, which regulate actin polymerization [14-16]. Rho kinase, also referred to as ROCK, is the major effector of RhoA and was discovered by a number of laboratories because of its ability to bind GTP-bound RhoA [17-19]. ROCK is a serine/threonine protein kinase of ~160 kDa which corresponds to gene products, ROCK I and ROCK II. The two ROCKs have spatially differential expressions. Rho kinase is autoregulated by its COOH-terminal domain, which folds back onto the active site to inhibit its kinase activity. Only the active GTP bound form of RhoA binds to ROCK and blocks the inactivation of the protein. As long as the active form of Rho is bound to ROCK, the kinase remains active [20]. Rho kinase can also be activated by arachidonic acid [21] and sphingosylphorylcholine [22]. The cleavage of the inhibitory COOH-terminus by caspases can result in an increase in ROCK activity during apoptosis [23]. The activation of ROCK results in the subsequent phosphorylation of a number of different downstream targets. The most well known target of Rho kinase is myosin light

chain (MLC) [24]. Myosin phosphatase is also phosphorylated by Rho kinase and this interaction causes an increase in phosphorylated MLC [25]. In addition, LIM kinases 1 and 2 are phosphorylated by ROCK and result in a stabilization of actin filaments. Many other proteins involved in actin cytoskeleton rearrangement are phosphorylated by ROCK, which explains why Rho GTPase activation can lead to such a profound alteration of actin cytoskeleton dynamics.

In addition to many studies that demonstrated the involvement of ROCK in actin rearrangements in cell culture system, genetically engineered mice are produced in attempts to better understand the role of the kinase *in vivo*. Although ROCK I and ROCK II knockout mice show distinct phenotypes, they share some common features. Both mouse lines demonstrate impairment in the development of the eyelid and ventral body wall. The majority of ROCK II knockout mice suffer from intrauterine growth retardation and fetal death due to defect(s) in the interaction of the embryo and placenta, therefore it is not due to any direct effects on the embryo's development. Both Rho kinase knockout mice are capable of maturing normally without developing major neurological defects [26, 27].

THE ROLE OF RHO/ROCK IN CARDIOVASCULAR AND PULMONARY DISEASES

The lack of direct embryonic defects with the deletions of ROCK I/II *in vivo* provides evidence for the viability of the use of ROCK inhibition to treat clinical problems. Rho kinase increases the MLC phosphorylation in smooth muscle by direct phosphorylation and also through the inhibition of myosin phosphatase. Therefore, ROCK is the first component of Rho GTPase pathway extensively characterized for its effect on vascular diseases. It was then suggested that the aberrations of RhoA/ROCK pathway play major roles in several disease states, particularly impacting on Ca^{2+} sensitization of smooth muscle in hypertension and possibly asthma as well as on cancer progression and neoangiogenesis [28]. It is now known that the vasopressor angiotensin II increases vascular tone by the activation of Rho GTPase [29, 30]. RhoA activates Rho kinase, which inhibits the expression of endothelial nitric oxide synthase (eNOS) [31]. The resultant decrease in nitric oxide (NO) causes vasoconstriction, which can lead to increased vascular resistance and hypertension. The connection among RhoA/ROCK activation, NO, and vascular tone is also validated by a NO deficient mouse model. Renal vascular tone is enhanced by an increased activation of RhoA/ROCK in eNOS knockout mice [32]. Bartter's/Gitelman's syndrome has been considered as a good human model to explore the mechanisms responsible for maintenance/control of vascular tone and vascular remodeling. Calo et al [33] discussed that in Bartter's/Gitelman's syndrome patients, RhoA/Rho kinase pathway is blunted as shown by the reduced gene and protein expression, reduced response to angiotensin II (Ang II) challenge of Rho kinase and PAI-1, and the reduced gene and protein expression of the upstream regulator of RhoA, p115RhoGEF [34, 35].

The connection between RhoA/ROCK activation and vasoconstriction has made the inhibition of the Rho GTPase pathway an appealing target for pharmacological treatment of hypertension, cerebral vasospasm, and vascular aneurysms. Two ROCK inhibitors (Fasudil and Y-27632, Fig 2) have been developed to test the efficacy of Rho pathway inhibition for the treatment of these diseases. Fasudil, [(5-Isoquinolinesulfonyl)homopiperazine, 2HCl], is the founding member of the isoquinoline series of Rho kinase inhibitors. It has a K_i of 330 nM for ROCK [36]. It is a cell-permeable Ca^{2+} antagonist [37, 38], which inhibits myosin light chain kinase ($K_i = 36 \mu\text{M}$) and also inhibits the Ca^{2+} sensitization of smooth muscle contraction [39]. Y-27632, [(R)-(+)-*trans*-N-(4-Pyridyl)-4-(1-aminoethyl)-cyclohexanecarboxamide, 2HCl], is a member of the 4-aminopyridine series of ROCK inhibitors, with a K_i of 220-300 nM [40,41]. The ATP binding site of ROCK can

accommodate the binding of fasudil or Y-27632 [42], which leads to the effective inhibition of its kinase activity.

Y-27632 is effective for the treatment of hypoxia induced pulmonary hypertension, which is an expected outcome due to effects of RhoA/ROCK activation in vascular remodeling (e.g. occlusive neointimal lesions in pulmonary arterioles) leading to the development of pulmonary hypertension [43-46]. Cerebral vasospasm can occur after a brain hemorrhage and results in a constriction of cerebral vasculature. Studies also indicate that Y-27632 is capable of causing vasodilation and reducing the amount of cerebral vasospasm [47]. Vascular aneurisms are caused by a weakening and thinning of blood vessels most frequently due to an increase in blood pressure against vessel walls. A mouse model of abdominal aortic aneurism found the ROCK inhibitor fasudil effective in the attenuation of the aneurism [48].

The success of the ROCK inhibitors in the treatment of vascular disease has encouraged the use of Rho pathway inhibition in clinical studies. In the early 1990's, Japanese researchers conducted many preliminary studies concerning the pharmacological properties of the ROCK inhibitor fasudil. Clinical trials utilizing the inhibitor after subarachnoid hemorrhage induced cerebral vasospasm have resulted in a significantly better outcome in fasudil treated patients when compared to the control group [49, 50]. The trial found no significant side-effects of the treatment, and the drug was approved for the treatment of cerebral vasospasm beginning in 1995 [51]. The use of fasudil has increased to include other cardiovascular problems such as angina pectoris and myocardial ischemia [52, 53]. Studies are currently underway to determine the efficacy of Rho GTPase inhibition in erectile dysfunction due to smooth muscle weakening [54-56]. Besides Fasudil and Y-27632, other newer selective RhoA/Rhokinase inhibitors (such as H-1152 and aminofurazan based inhibitors, see Fig 2) also show vasodilatory and antihypertensive effects [57, 58]. Most recently, H-1152 studies in vascular smooth muscles showed that phorbol-12, 13-dibutyrate induces Ca^{2+} -independent contractions by inhibiting myosin light chain phosphatase (MLCP) through activation of GTP-RhoA and subsequent phosphorylation of MYPT1 and CPI-17 [59]. H-1152 is a cell-permeable isoquinolinesulfonamide compound that acts as a highly specific, potent, and ATP-competitive inhibitor of Rho kinase ($K_i = 1.6$ nM) [60, 61].

Although signaling through the Rho GTPase pathway is significantly involved in the maintenance of vascular tone, the implications for the therapeutic use of Rho pathway inhibition is not limited to the cardiovascular diseases discussed above [62]. RhoA/ROCK activation has been implicated in the induction of NAD(P)H induced oxidative stress [63] and also nuclear factor kappa B (NF- κ B) activity [64, 65]. Both processes are important in the initiation of vascular inflammation and remodeling responsible for atherosclerosis. In addition to cardiovascular disease, Rho kinase inhibitors have emerged as potential intervention in airway diseases such as allergen-induced acute bronchoconstriction, airway hyperresponsiveness, and inflammation (see recent reviews at [66-68]).

During smooth muscle contraction and relaxation, inhibition of sustained smooth muscle contraction by cAMP- and/or cGMP-dependent protein kinase (PKA and PKG) is preferentially mediated by phosphorylation of RhoA. In this case, the phosphorylation is accompanied by translocation of membrane-bound RhoA to the cytosol and complete inhibition of agonist-stimulated Rho kinase and phospholipase D activities, RhoA/Rho kinase association, MLC(20) phosphorylation, and Ca^{2+} sensitization of contraction in vascular smooth muscle. All of these effects indicate a direct regulation by phosphorylation at RhoA level [69, 70]. RhoA/ROCK-mediated inhibition of the PI3K/Akt pathway is also involved in alterations in glucose transport and insulin resistance [71]. Therefore, the Rho

pathway through ROCK is involved in several fundamental cardiovascular and pulmonary processes and has potential therapeutic use in these disease states.

RHO PATHWAY MODULATION IN NEUROLOGICAL DISEASES

In recent years, intense focus has been given to developing therapeutic strategies involving Rho pathway inhibition for the treatment of neurological diseases. Many studies have indicated that Rho pathway inhibition is beneficial for the promotion of recovery following spinal cord injury [72-77]. These studies have firmly established that Rho GTPases are involved in nerve regeneration and called for accelerated efforts to further investigate their involvement in nerve injury. In fact, the activation of Rho GTPases has been implicated in the promotion of nerve degeneration following spinal cord injury and traumatic brain injury [78-81]. On the other hand, the effects of Rho inhibition directly on regeneration of neurons have been clearly demonstrated in cell culture using molecular and cellular biology methods [82, 83]. In the in vitro and animal models, inhibitors of the Rho pathway have also been demonstrated to be effective in the treatment of stroke, inflammatory reactions, Alzheimer's disease, and neuropathic pain [84, 85]. Although these findings show a wide array of possible therapeutic implications for Rho GTPase in neurological diseases, the consensus seems to be that the current knowledge of this area may still need further development to promote mechanism-based Rho pathway inhibitor intervention for neurological disorders.

It is possible that the versatility of Rho pathway inhibitors in neurological diseases stems from the involvement of the pathway in inflammation. Nerve tissue injury results in an inflammatory reaction that hinders the regeneration of affected neurons. Studies have proposed that RhoA and ROCK activation are essential components of the inflammation cascade. Neuroinflammation results from the migration of leukocytes into the nervous tissue. The migration of activated leukocytes is controlled by Rho GTPase dependent CD54 activation and in a model of multiple sclerosis, leukocyte invasion could be limited by Rho inhibition [86]. Several studies report that functional endothelial T cell migration is mediated through the Rho signaling pathway [86-88]. Another study reports that the transendothelial migration of monocytes is upregulated by RhoA activation and the CNS can be protected from this reaction using the ROCK inhibitor Y-27632 [89]. In animal models of spinal cord injury, Rho activity is significantly increased at the site of injury. The increase in Rho activity strongly correlates with a decrease in the functional recovery of the animal. By applying a Rho pathway inhibitor to the site of injury, the recovery of the animals is greatly improved. These studies suggest that the secondary damage caused by inflammation at the site of injury can be alleviated with Rho pathway inhibition. Therefore, the reduction in inflammation may be necessary for nerve cells to survive and regain function [72, 74, 78, 81].

The importance of the anti-inflammatory component of Rho pathway antagonists can also be seen in Alzheimer's disease research. Neurofibrillary tangles and amyloid plaques are hallmark characteristics of the disease [90]. It is still not understood whether the tangles and plaques are the cause of the disease or merely the result of its progression. Major efforts in the neuroscience research community have been geared toward elucidating the cause of the plaque formation in the brains of Alzheimer's disease patients [91, 92]. We now know that the plaques are the result of an increase in A_β levels, which aggregate to form the core of amyloid plaques. It was recently discovered that non-steroidal anti-inflammatory drugs (NSAIDs) can be used to lower A_β 40/42 levels. The NSAIDs used are well known cyclooxygenase (COX) inhibitors, although the drugs are effective independent of the resultant COX activity. A study showed that the NSAIDs were able to lower A_β 40/42 levels by reducing the activation of the Rho GTPase pathway [93]. Other studies indicated that Rho kinase inhibition alone does not elicit sufficient effects on A_β 42 level [94], the most

potent A β form in A β fibril formation in the plaque. On the other hand, Alzheimer's related neurotoxic peptides can also act upstream of Rho GTPases pathway to alter synaptic plasticity and disturb synaptic function in Alzheimer's disease [95]. In some cases, the A β oligomers activate ROCK and inhibit Rac1/Cdc42 downstream target p21-activated kinase (PAK), leading to dendritic spine retraction, synaptic collapse, and the loss of synaptic plasticity [96].

Novel Rho pathway inhibitors are continually discovered, as there is increasing need for their potential applications in neurological disorders. Recently, our laboratory has published the results of the use of a p75^{NTR} ligand mimetic Rho inhibitor capable of enhancing regeneration following neuronal injury [83]. Signaling through p75^{NTR} is responsible for a wide array of downstream signaling events culminating in either an increase in apoptosis or survival [97]. The LM11A series compounds are designed as mimetics of nerve growth factor (NGF) loop 1, the p75^{NTR} binding domain. Several compounds are isolated and tested with four compounds demonstrating activity through receptor binding. These compounds are LM11A-7, 24, 28, and 31 (Fig 3), and they exhibit EC₅₀ of 100~300 pM. The compound LM11A-31 has been shown to promote survival signaling at 5 nM in primary neuronal cultures [98]. The receptor activation also seems to lie upstream of Rho GTPase pathway and has been shown to be involved in the modulation of Rho GTPase activity. The activation using the compound LM11A-31 significantly lowers the amount of GTP bound RhoA in rat cortical neurons [83]. Remarkably, these p75^{NTR} ligand mimetics inhibit A β -induced neuritic dystrophy, A β -induced activation of molecules involved in Alzheimer's disease pathology, and tau phosphorylation [99]. They block A β -induced hippocampal long-term potentiation (LTP) impairment [99]. The amino acid derivative LM11A-31 is capable of oral uptake and has a structure compatible with potential blood brain barrier penetration.

LM11A-31 has been used in a recent study investigating the effects of anti-neoplastic agents on the nervous system. One anti-neoplastic agent used, cisplatin, can produce a dose-limiting neurotoxicity with clinical treatment. Cisplatin treatment causes a significant decrease in the number and length of dendrites in hippocampal and cortical primary neurons. Importantly, the inhibition of RhoA signaling using the compound LM11A-31 is able to reverse neurodegeneration caused by cisplatin [83]. This study indicates the effectiveness of p75^{NTR} mediated Rho pathway inhibition on chemotherapy induced neurotoxicity. Therefore, p75^{NTR} related inhibitors of Rho GTPase pathway may also prove to be beneficial in other models of neuronal injury and diseases.

RHO SIGNALING IN CANCER

Over the past decade, an increasing number of studies have found a correlation between Rho protein overexpression and cancer incidence [5]. The Rho GTPase pathway is involved in the regulation of a wide variety of cell processes such as cell morphology, survival, proliferation, and adhesion. These biological processes are important in the deregulation of cell signaling that results in transformation and metastases. The Rho family of small GTPases is unlike the Ras protein in that there is no evidence of genetic mutations in the Rho proteins that would result in a tumorigenic phenotype. However, Rho GTPase protein overexpression occurs in a large number of cancers.

One breast cancer study has compared the expression of RhoA, Rac1, and Cdc42 in tumor and normal tissue from patients diagnosed with grade III ductal carcinoma. The study found an overexpression of all three Rho proteins in 100% of the tumor samples, when compared to the normal tissue controls. The study also indicates a correlation between Rho protein expression and tumor malignancy [100]. Testicular germ cell tumors overexpress both RhoA and Rho kinase and the expression is indicative of cancer progression [101]. Similar

findings have been gathered from studies involving lung, ovarian, gastric, liver, and bladder cancers. Many other types of cancers display an increase in Rho activity. Studies indicate that the proliferative properties associated with these cancers are partly due to the overexpression of Rho proteins. In the case of breast cancer, the cells express CD44, which promotes cell survival and growth. There is evidence that RhoA is a downstream target of CD44 activation that results in an increase in the metastatic potential of cancer cells. Inhibition of Rho signaling by overexpression of a dominant negative form of Rho kinase blocks CD44 mediated progression of breast cancer [102]. The Rho kinase inhibitor Y-27632 should produce similar effects as the dominant negative Rho kinase. At least one study has showed that in lung carcinoma A549 cells, Y-27632 enhances cisplatin-induced cytotoxicity through suppression of focal adhesion kinase-independent mechanism [103].

Recently, commonly used statin drugs (Fig 4) have been shown to cause a decrease in Rho activity, which inhibits the progression of several cancer types [104]. Statins inhibit hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase. HMG-CoA is essential for cholesterol production, and HMG-CoA reductase inhibitors (such as statins) are frequently used to treat hypercholesterolemia. The Rho inhibitory activity of statins results from the inhibition of farnesyl pyrophosphate and geranylgeranyl pyrophosphate synthesis, which are responsible for RhoA prenylation. Carboxyl terminal prenylation of RhoA is necessary for the proper membrane localization and activation of the protein [105].

Cerivastatin, or Baycol/ Lipobay { (E,3R,5S)-7-[4-(4-fluorophenyl)-5-(methoxymethyl)-2,6-dipropyl-pyridin-3-yl]-3,5-dihydroxy-hept-6-enoic acid } can reduce RhoA activity in MDA-MB-231 breast cancer cells, limiting the motility and migration of the cancer [106]. Atorvastatin [(R-(R*, R*))]-2-(4-fluorophenyl)-beta, delta-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid), marketed as Lipitor, inhibits Rho activity by reducing Rho geranylgeranylation. It has been shown to inhibit metastasis of human melanoma cells both *in vitro* and *in vivo* [107]. Lovastatin, or Mevacor ([8-[2-(4-hydroxy-6-oxo-oxan-2-yl)ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl]-2-methylbutanoate) inhibits Rho-mediated expression of E-selectin, which significantly reduces tumor cell adhesion [108, 109]. It was found that although lovastatin increases the expression of RhoA, RhoB and RhoC in human erythroleukemia (HEL) cells, these Rho proteins are accumulated as unprenylated form; therefore, they are not favorable for membrane localization and activation [109]. The decrease in tumor invasiveness demonstrated by statins is similar to the reduction seen with the ROCK inhibitor Y-27632 [110]. The statin Simvastatin [(1S,3R,7R,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxo-oxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl]-2,2-dimethylbutanoate), marketed as Zocor, inhibits RhoA/ROCK activation and increases the sensitivity of human colon cancer HT29 cells to doxorubicin treatment. A recent study also provides evidence for the benefit of adjuvant Rho pathway inhibition in cancer treatment [111].

Currently, there are relatively few drugs or pharmacologic tools that directly target Rho GTPases despite the effort and the accumulating structural information on the specific Rho-RhoGEF, Rho-RhoGAP, or Rho-RhoGDI interaction [112-116]. Much of the effort to date has focused on inhibiting the carboxyl terminal isoprenylation of the Rho GTPases. The most widely used inhibitors of this modification include farnesyltransferase and geranylgeranyltransferase inhibitors and the cholesterol-lowering statin drugs as described above. However, these compounds are not specific for the Rho family of small GTPases so their effects are difficult to interpret mechanistically. To date, the only specific and direct inhibitor of Rho GTPases is the Rac1 inhibitor NSC23766 [117]. This first-generation small-molecule inhibitor of Rac1 (Fig 5) is identified by a structure-based virtual screening of compounds that fit into a surface groove of Rac1 known to be critical for GEF binding.

NSC23766 effectively inhibits Rac1 binding and activation by the Rac-specific GEF Trio or Tiam1 without interfering with the closely related RhoA or Cdc42 binding or activation by their respective GEFs. NSC23766 can also inhibit serum or platelet-derived growth factor-induced Rac1 activation and lamellipodia formation characteristic of Rac1 activation. When applied to human prostate cancer PC-3 cells, NSC23766 inhibits the proliferation, anchorage-independent growth and invasion phenotypes that require the endogenous Rac1 activity. Therefore, NSC23766 constitutes a Rac-specific small-molecule inhibitor that could be useful to study the role of Rac in various cellular functions and to reverse tumor cell phenotypes associated with Rac deregulation [117]. However, NSC23766 is not very potent ($IC_{50} \sim 50 \mu M$), and other specific inhibitors directly targeting RhoA and Cdc42 are lacking. Thus, there is a clear need and opportunity for specific inhibitors of Rho GTPase signaling pathways.

In a recent study, Evelyn et al [118] took advantage of a modified serum response element (SRE)-luciferase reporter to undertake a high-throughput screen aiming to identify novel small molecule inhibitors of the RhoA family signaling pathway. By this approach, two novel and structurally similar small-molecule inhibitors have been identified, which suppress RhoA-stimulated transcription. Mechanistic analyses show that the more potent compound, CCG-1423 (see Fig 5) elicits its effects downstream of RhoA and actin polymerization by a mechanism targeting MKL/SRF-dependent transcriptional activation. Interestingly, CCG-1423 inhibits lysophosphatidic acid (LPA) receptor-stimulated DNA synthesis, cell growth, cell survival, and Matrigel invasion for several cancer cell lines [118].

RNA interference of Rho has also proved to be quite effective in combating a number of different cancer types. In human gastric carcinoma, RhoA and RhoC RNA interference inhibits the proliferation and aggressiveness of the cancer [119]. Similar results have been obtained in MDA-MB-231 breast cancer cells in vitro and in vivo [120]. Melanoma cells can evade immune system detection by FasL mediated apoptosis of leukocytes. A study using mouse melanoma cells found that the reduction of RhoA expression using siRNA was able to regulate leukocyte apoptosis [121]. The regulation by non-coding RNAs such as microRNA (miRNA) represents new possibility of targets involved in cancer progression. It is anticipated that miRNAs targeting Rho GTPase pathway (e.g. miR-29) will provide valuable information for the development of novel cancer therapeutics [122]. The positive results of direct Rho GTPase inhibition further emphasize the potential impact of Rho GTPase pathway inhibition for the treatment of cancer [123].

CONCLUSIONS

Due to the emergence of evidence implicating Rho GTPase involvement in a host of disease states, signaling through Rho GTPases is becoming a promising target for pharmacological intervention. The ROCK inhibitor fasudil is currently used in the treatment of cerebral vasospasm; however, the need to increase the application and bioavailability of Rho pathway inhibitors continues to rise. The discovery of novel Rho pathway modulators, like LM11A-31, NSC23766, and CCG-1423, represent important milestones for the regulation of the Rho pathway using associated signaling molecules. The continued exploration into the function of Rho GTPases is vital to the development of medicinal intervention of cardiovascular disease, neurological disorders, and cancer progression.

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LIST OF ABBREVIATIONS

COX	Cyclooxygenase
CPI-17	Protein-kinase C-potentiated myosin phosphatase inhibitor
eNOS	endothelial Nitric Oxide Synthase
GAP	GTPase Activating Protein
GDI	Guanine Nucleotide Dissociation Inhibitor
GDP	Guanosine 5 -diphosphate
GEF	Guanine Nucleotide Exchange Factor
GTP	Guanosine-5 -triphosphate
HMG-CoA	3-hydroxy-3-methyl-glutaryl-Coenzyme A
miRNA	MicroRNA
MLC	Myosin Light Chain
MLCP	Myosin light chain phosphatase
MYPT1	Myosin phosphatase target subunit 1
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NF B	Nuclear Factor kappa B
NGF	Nerve Growth Factor
NSAID	Non-Steroidal Anti-Inflammatory Drug
PI3K	Phosphoinositide-3-Kinase
ROCK	Rho associated protein kinase
SRE	Serum response element

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Fig. (1). Schematic illustration of Rho GTPase signaling pathway. A. Receptor dependent and independent signaling through Rho GTPase pathway leading to actin reorganization. Numbered arrows indicate where the inhibitor targets are in the Rho GTPase signaling cascade: 1, Y27632, fasudil, and H-1152; 2, LM11A31; 3, statins; 4, NSC23766; 5, CCG-1423. B. Regulation of Rho GTPase activation and inactivation through Rho-GEFs, Rho-GAPs, and RhoGDIs.

Fig. (2).
Examples of Rho kinase (ROCK) inhibitors, fasudil, Y-27632, and H-1152.

Fig. (3).
LM11A compounds exhibiting activity upon p75^{NTR} binding: LM11A-7, 28, 24, and 31.

Fig. (4).
Selection of statin drugs capable of modulating Rho pathway signaling.

NSC23766

CCG-977

CCG-1423

Fig. (5).
Novel inhibitors of RhoGTPase pathways, NSC23766, CCG-977, and CCG-1423.