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REVIEW

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RhoA-ROCK2 signaling possesses complex pathophysiological functions in cancer progression and shows promising therapeutic potential

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Abstract

The Rho GTPase signaling pathway is responsible for cell-specific processes, including actin cytoskeleton organization, cell motility, cell division, and the transcription of specific genes. The implications of RhoA and the downstream effector ROCK2 in cancer epithelial-mesenchymal transition, migration, invasion, and therapy resistance associated with stem cells highlight the potential of targeting RhoA/ROCK2 signaling in therapy. Tumor relapse can occur due to cancer cells that do not fully respond to adjuvant chemoradiotherapy, targeted therapy, or immunotherapy. Rho signaling-mediated mitotic defects and cytokinesis failure lead to asymmetric cell division, allowing cells to form polyploids to escape cytotoxicity and promote tumor recurrence and metastasis. In this review, we elucidate the significance of RhoA/ROCK2 in the mechanisms of cancer progression and summarize their inhibitors that may improve treatment strategies.

Keywords RhoA, ROCK2, Cancer stem cell, Asymmetric cell division, Inhibitor

Introduction

Rho GTPases are the members of the Ras superfamily and are known for their important roles in regulating the actin cytoskeleton. Rho, Rac, and Cdc42 are the most extensively characterized Rho GTPases. The Rho subfamily contains the three isoforms RhoA, RhoB, and RhoC [1]. The Rho family proteins have lipid modifications that allow them to localize to cell membranes and act as molecular switches, cycling between active GTP-bound and inactive GDP-bound forms. Binding to GTP is promoted by Rho guanine nucleotide exchange factors (Rho GEFs), and they can interact with effectors or target molecules in the GTP-bound form to initiate downstream responses, whereas GTP hydrolysis is catalyzed by Rho GTPase-activating proteins (Rho GAPs), reverting the proteins to the GDP-bound state to complete the cycle and terminate signaling. Rho GDP-dissociation inhibitors (GDIs) sequester GDP-bound Rho GTPases in the cytoplasm and inhibit GDP and GTP exchange activity [1–3].

Rho proteins play critical roles in actin cytoskeleton remodeling, influencing cell motility and supporting cellular processes such as migration, invasion, and tube formation. RhoA is involved in gene transcription, cell cycle progression, and cell transformation and RhoA plays a

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crucial role in the progression and development of many malignancies, such as breast, gastric, and colorectal cancer [4]. It regulates cancer cell survival, proliferation, adhesion, the cell cycle, and gene transcription [5, 6]. Mutations in RhoA have been reported in many human cancers, wherein they contribute to the malignant processes [7]. RhoA is overexpressed in many tumors and has been associated with poor prognosis [8], and its inhibition results in decreased tumor proliferation and migration [9]. Upregulated RhoC also regulates several tumor phenotypes [10, 11] and induces TGFβ signaling mediated epithelial-mesenchymal transition (EMT) in ovarian and cervical cancers [12, 13]. In contrast, RhoB has the opposite function, acting as a negative modifier or suppressor gene in cancer cells. In a variety of solid tumors, such as lung, head and neck, and brain cancers, RhoB levels decrease as tumor progresses [14].

Activated RhoA triggers one of its downstream effectors Rho-associated coiled-coil-containing protein kinases, ROCK1 or ROCK2. These kinases play a vital role in stress fiber and focal adhesion formation, smooth muscle contraction, neurite retraction, microvilli formation, and cell migration. ROCK2 also plays an important role in tumor progression. In this review, we discuss the role of RhoA/ROCK2 signaling in normal cells and its correlation with cancer cell invasion, migration, division, tumor stemness, and resistance to therapy. This study aimed to understand tumor progression and identify potential targeting strategies.

Structure of Rho proteins

The gene encoding RhoA (former gene name: aplysia Ras-related homolog, ARH12) is located on chromosome 3 in humas, and its precursor mRNA is subject to highly variable alternative splicing, resulting in seven known transcript variants for RhoA, which are then translated into six different RhoA isoforms [15]. The protein structures of Rho subfamily members have been intensively studied for decades, revealing their binding to different nucleotides or nucleotide analogs at different activity states and their interactions with different effectors, including GEFs, GAPs, and GDIs.

RhoA, RhoB, and RhoC possess a relatively conserved N-terminal portion containing a G domain (also known as RhoA-like domain) and a less-conserved C-terminal tail. The conformational changes from activated to inactivated states are mainly restricted to two surface loop regions: switch I and switch II [16]. The G domain consists of five conserved sequence motifs (G1 to G5) that are involved in nucleotide binding and hydrolysis [17]. The conformational changes in switches I (G2) and II (G3) from the inactive GDP-bound to the active GTP-bound state are also a prerequisite for the GTPase to bind to effector proteins. This possibly occurs via interactions with hydrophobic residues in switch regions that are exposed in the active state of the GTPase [18]. In addition to the switch regions, an inserted α-helix (located between the G4 and G5 motifs) has been shown to be important for effector protein interaction [19]. The crystal structures of the GTPase-binding domains (GBDs) of the RhoA-associated coiled-coil kinase (ROCK)-RhoA complexes revealed that, as predicted from their primary structure, these domains formed α-helical coiled coils arranged in a parallel fashion. A 13-residue left-handed coiled-coil in the C-terminal portion of the ROCK-GBD binds exclusively to the switch and α2 regions of RhoA and is considered the minimal sequence required for Rho-interacting motif activity [20].

The C-terminal hypervariable region terminates with a common sequence known as CAAX (where C represents cysteine, A is an aliphatic amino acid, and X is any amino acid), and post-translational modifications in this sequence, including prenylation, endoproteolysis, and carboxyl methylation, are critical for the subcellular localization of Rho GTPases [21] (Fig. 1). The X residue determines which isoprenoid will be added to the cysteine. If the amino acid in the X position is leucine, isoleucine, or phenylalanine, as in the Rho/Rac family

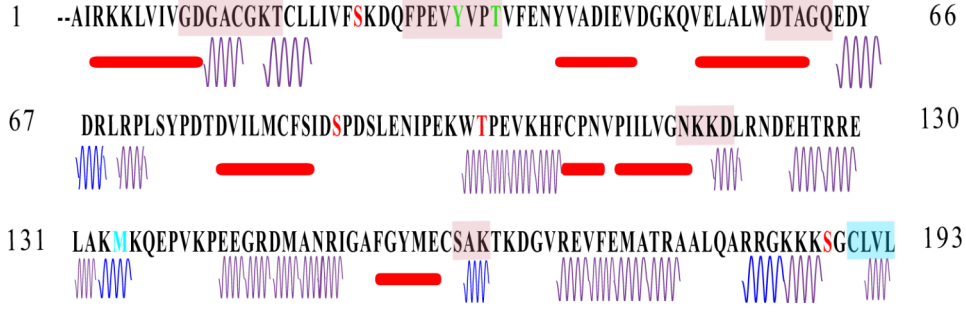

Fig. 1 Sequential schematic of the protein structural elements and binding sites of RhoA as retrieved from the PDB database (ID number: 1s1c). Different colors of a single letter represent different post-translational modification sites: red for phosphoserine, green for glycosylation sites, and blue for ubiquitination sites. The red-shaded background on the sequence represents the site that binds to GTP, and the blue-shaded background is the CAAX sequence that GDI interacts with. The purple curve line and red boxes below the sequence correspond to α-helices and β-strands, respectively

of proteins, geranylgeranyltransferase (GGT) modifies CAAX Cys residues, while farnesyltransferase (FT) utilizes mostly serine, methionine, or as other amino acids in the X position [22, 23]. Prenylation is followed by truncation and methylation and the lipid moiety makes the C-terminus hydrophobic and helps anchor it to membranes [24]. In contrast to RhoA and RhoC proteins, both farnesylated and geranylgeranylated forms of RhoB (RhoB-F and RhoB-GG) are present in COS and Rat1/ras cells [25–27].

Post-translational modifications of RhoA

To ensure appropriate spatiotemporal activation, the GTPases undergo post-translational modifications. The most well-studied are the phosphorylation of serine, threonine, and tyrosine residues by several critical kinases [28]. RhoA is phosphorylated by cAMP-dependent protein kinase (PKA), cGMP-dependent protein kinase (PKG), and protein kinase C (PKC) on Ser 188 [29], whereas ste20-related kinase (SKL) and AMP-activated protein kinase subunit alpha 1 (AMPKα1) inactivate Ser188 [30, 31]. Phosphorylation of Ser188 deactivates RhoA by increasing its interaction with Rho GDI, leading to its translocation from the site of action at the membrane to the cytoplasm [32] and causing the collapse of actin stress fibers. Extracellular signal-regulated kinase (ERK) phosphorylates RhoA at Ser88 and Thr100 to upregulate RhoA [33]. Mammalian Ste20-like kinase 3 (Mst3) phosphorylates RhoA on Ser26 to inactivate RhoA [34]. In addition, other tyrosine residues, Tyr34, Tyr66, and Tyr42, are regulated by different tyrosine kinases such as Bcr-Abl, Src, and c-Met [35–37]. Additionally, RhoA undergoes ubiquitination, leading to various outcomes, such as re-localization or degradation [38]. RhoA is ubiquitylated by E3 ubiquitin ligase complexes, such as SMAD-specific E3 ubiquitin protein ligase (SMURF1), Cullin3, and RNF8, leading to proteasomal degradation and regulation of protein turnover [39–42]. For RhoA, the prerequisite process involves the addition of a geranylgeranyl moiety to Cys190 in the CAAX motif, followed by proteolysis and methylation [21, 43].

Role of RhoA in cytoskeletal rearrangement

Cytoskeletal rearrangements play critical roles in the regulation of various cellular processes linked to transformation, including proliferation, contact inhibition and apoptosis [44]. When bound to GTP, RhoA interacts with many downstream effectors. ROCK and mammalian diaphanous-related protein (mDia) initiate a network of cytoplasmic and nuclear signaling cascades. mDia facilitates actin nucleation and polymerization and induces long, straight actin filaments [45, 46], and stabilizes and aligns microtubules in interphase cells [47]. Rho acts on ROCK to phosphorylate myosin phosphatase and myosin light chain kinase at Ser19 [48, 49], leading to actin filament formation and increased actin-myosin interactions [50, 51] and contribution to the invasive phenotype [52]. ROCK phosphorylates LIM kinase 1 (LIMK1) at Thr508 [53], then phosphorylates and inactivates cofilin (filament severing and depolymerization from the pointed end) to regulate actin filament stabilization, focal adhesion, and actin network assembly [54, 55].

RhoA-ROCK signaling is involved in the cell cycle

Rho proteins regulate cell cycle proteins that are mainly involved in the G1/S transition [56, 57]. Activation of RhoA and Rho-dependent stress fiber accumulation upregulates cyclin D1 expression levels and consequent G1-phase progression. Macrophage migration inhibitory factor promotes the activation of the canonical extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase cascade and cyclin D1 expression by stimulating Rho GTPase activity and downstream signaling to stress fiber formation and subsequent progression through G1 to S phase [58–61]. In addition, RhoA increases the ubiquitination-dependent degradation of p27 (cyclin-dependent kinase inhibitor $p27^{kip1}$) by contributing to an earlier stage of G1 progression [62]. Inhibition of RhoA and ROCK resulted in increased p21 (cell cycle inhibitor $p21^{Cip1/Waf1}$) expression, which is dependent on phosphorylated ERK, resulting in decreased cell proliferation [63, 64].

RhoA regulates mitosis

Mitotic progression is highly regulated and involves the dynamic modulation of cell shape and morphology, primarily through the remodeling of the actin and microtubule cytoskeleton. Rho GTPases in animal somatic cells regulate the mitotic stages from prophase to telophase, and then to cytokinesis [57, 65–67]. During prometaphase, chromosomes are captured at kinetochores as mitotic spindles assemble. The presence of all chromosomes at the spindle equator is necessary for accurate chromosome segregation; however, this process is prone to error [68].

Cytokinesis is a finely orchestrated process that requires the formation and progression of a cleavage furrow generated by the contraction of an actomyosin contractile ring anchored to the plasma membrane by cytoskeletal proteins [69]. At the onset of mitosis, actin cytoskeleton rearrangement, de-adhesion, and an increase in cortical rigidity accompany mitotic cell rounding, during which a flat cell becomes spherical [70]; Cdk1/cyclin B1 mediates the phosphorylation of RhoA regulators causing a global increase in RhoA activity; activated RhoA acts on ROCK to alter cell shape [71]. This is followed by nuclear envelope rupture during mitosis and an increase in RhoA activity and the concentration of active RhoA in the cell

cortex. Following chromosome segregation, cells immediately undergo highly ordered cytoplasmic division, and RhoA plays a central role in regulating cytokinesis [72]. RhoA activation is required to stabilize midzone microtubules and maintain midzone structures after anaphase onset or during cytokinesis [73]. Recruitment of RhoA determines the cleavage sites and promotes contractile ring assembly and furrow ingression [74, 75] or induces polyploidy if actin filaments are not concentrated at furrow sites [76, 77]. During cleavage, daughter cells move apart to expose the intercellular bridges that stretch between them. Cytokinesis is completed when the intracellular bridge is severed.

Intermediate filament (IF) proteins are located in the cleavage furrow and must be phosphorylated by ROCK during late cytokinesis, which locally breaks down IFs and separates IF networks efficiently [78, 79]. If cytoplasmic division fails and intermediates are formed in this state, nascent daughter cells may remain attached. This results in the generation of binucleated aneuploid or polyploid giant cells that may lead to genomic instability and contribute to the initiation and/or progression of tumorigenesis. Megakaryocytes (MKs), which are naturally polyploid cells that give rise to platelets, require RhoA/ROCK/F-actin for cytoplasmic maturation, and inhibition of this pathway leads to macrothrombocytopenia [80–82].

RhoA-ROCK2 pathway and asymmetric cell division (ACD)

The RhoA-ROCK2 signaling pathway plays a crucial role in cytoskeletal rearrangement and regulation during somatic cell division. Deviations in this pathway can result in aneuploidy formation or ACD, which can damage various cellular processes such as plasma membrane invagination, cleavage site positioning or specification, cleavage furrow formation, ingression, midbody formation, and cell separation [65, 69, 83]. Aneuploidy is a common characteristic of cancer cells present in approximately 90% of solid human tumors and 50% of hematopoietic cancers. Hypoxia stimulates the NPY/Y5R axis and leads to RhoA overactivation, cytokinesis defects, and polyploidy, triggering chromosomal instability and bone metastasis in Ewing sarcoma [84] (Fig. 2).

Dynamic contractile ring control mitosis through RhoA recruitment effectors

The contractile forces required for furrow ingression are provided by a ring of filamentous actin and myosin II, which are juxtaposed with the cell membrane at the equator of the dividing cells. RhoA-dependent ROCK activity is required for myosin II recruitment to the cortex, whereas myosin light chain kinase (MLCK) activity promotes myosin II turnover [85]. Uniform tension distribution around the ring depends on continuous turnover of actin filaments; however, a nonuniform tension distribution around the ring leads to peeling off of actin and myosin [86]. Cofilin is activated by myosin II to polymerize actin and assemble the contractile ring [87], rapidly accumulated during the late stages of furrowing, and eventually enriched at the midbody [88]. Cofilin, an actin-binding protein, contributes to turnover by severing actin filaments for normal function. The absence of cofilin results in longer filaments, higher ring tension, and increased actin dissociation from myosin in fission yeast [89].

Furthermore, F-actin is also required for anchoring the mitotic spindle to the cell cortex and determining the direction of spindle movement [90]. It is plausible that in the absence of cofilin, the excessive amount of actin forms filaments during mitosis that prevent proper spindle positioning and manifest as chromosome segregation defects and aberrant cytokinesis [91]. After silencing endogenous cofilin, cells lacking nuclear localization fail to sever nuclear F-actin during mitotic exit, resulting in defective nuclear volume expansion and chromatin decomposition [92].

Rho GEFs and GAPs involved in ACD through act on RhoA

Rho GAPs such as IQGAP [93–95], ARHGAP19 [96, 97], and ARHGAP11A [98], affect the recruitment and localization of key cytokinesis, or involved in spindle orientation. MgcRacGAP (RACGAP1, named Cyk-4 in *C. elegans*, RacGAP50C in *Drosophila* [99, 100]) localizes to the central spindle and contractile ring and binds the kinesin-like protein MKLP-1 to form an evolutionarily conserved complex called centralspindlin. It is essential not only for cytoplasmic division of somatic cells but also for cytoplasmic contraction, leading to intercellular bridges [101]. Rho GEF Ect2 localization to the central spindle depends on centralspindlin [102]. Ect2 depletion impairs microtubule attachment to kinetochores and causes prometaphase delay and abnormal chromosome segregation [103]. Rho GEF-H1/Lfc associates with mitotic spindles and spindle microtubules. Disruption of Lfc or the central spindle results in a reduction of active RhoA concentration in the equatorial plane, which in turn leads to a delay in mitosis [104–106]. MP-GAP (Rho GAP) was shown to target RhoA during mitosis/cytokinesis, and depletion caused excessive RhoA activation in M-phase, leading to the uncontrolled formation of large cortical protrusions and late cytokinesis failure [107].

Proper completion of cytokinesis requires the interplay of Rho GEFs and GAPs to appropriately regulate RhoA levels. The GRAF (Rho GAP) and RhoGEF2 are in balance for proper activation of actomyosin ring contraction [108]. Endogenous p190RhoGAP colocalize with Ect2 at the cleavage furrow during cell division [109]. Cells with

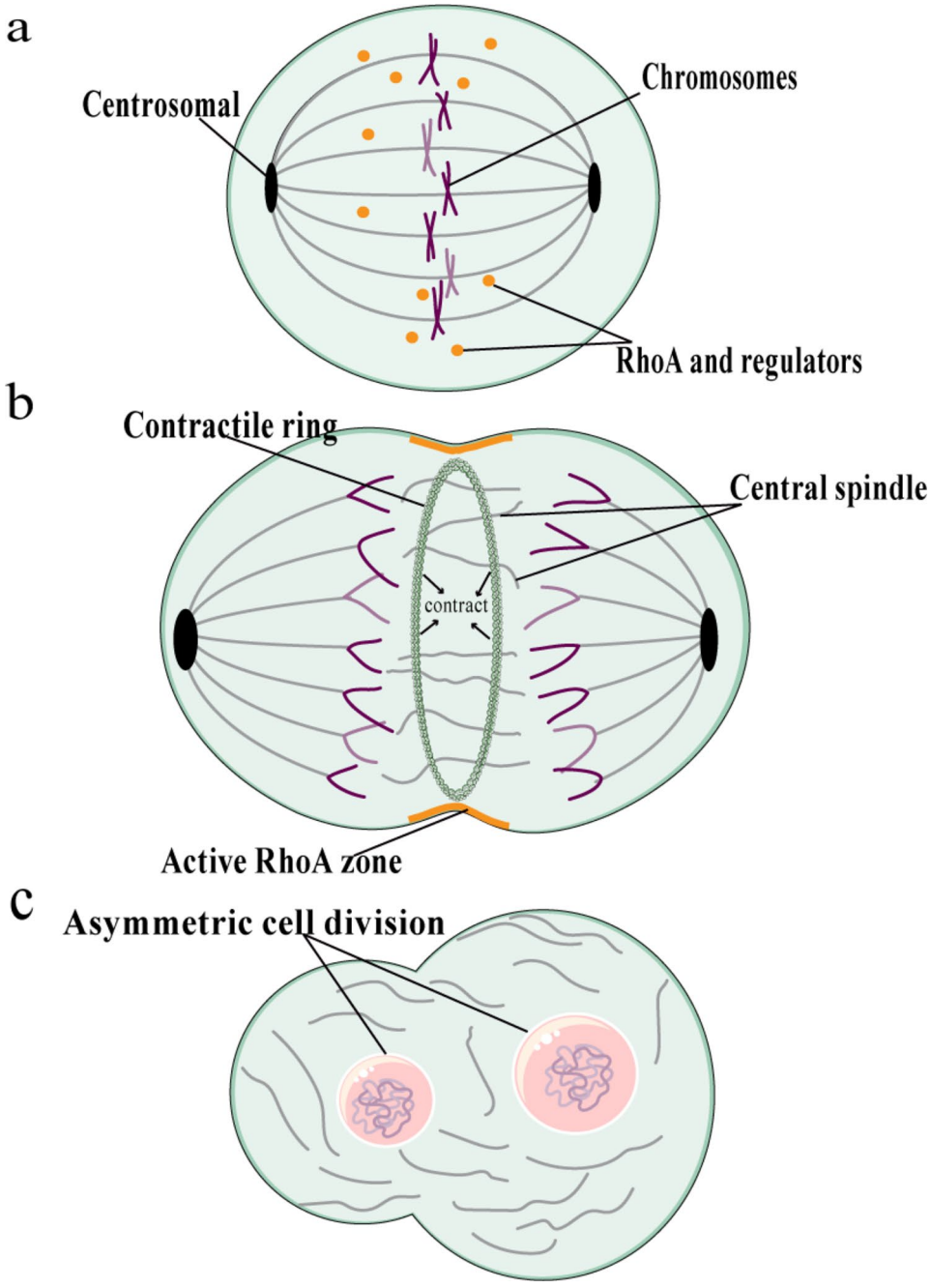

Fig. 2 RhoA signaling pathway regulates cell asymmetric division. (**a**) Chromosomes are arranged on the equatorial plate with two levels of centromeres pulling them laterally. (**b**) The central spindle recruits the Rho GEF and delivers it to the plasma membrane where it activates RhoA. Activated RhoA directs the assembly of the contractile ring containing the filamentous actin myosin II and contracts to change cell shape. (**c**) Failure of cell division owing to lack of contractility or centrosome can lead to asymmetric cell division

ectopic or loss of expression of the protein have reduced Rho GTP levels at the cleavage furrow and become multinucleated or fail to divide [110].

RhoA-ROCK signaling involved in ACD through centrosome disorganization

The centrosome is a small, self-replicating organelle that coordinates with mitosis [111, 112]. Abnormalities in centriole duplication, disengagement, or loss are hallmarks of cancer [113, 114], these abnormalities cause delays in the bipolar spindle assembly, high rates of chromosomal instability, and aneuploidy in vertebrate somatic cells [115, 116]. Signaling components, such as kinases and phosphatases, including ROCK, are associated with centrosomes, spindle poles, and microtubule organizing centers (MTOCs) [117]. They could maintain

centrosome stable and separation. ROCK2, associated with nucleophosmin (NPM), prevents aberrant centrosome amplification and a high frequency of multinucleated cells [118, 119]. Inhibition of ROCK1/2 affects chromosome segregation, bypasses the spindle assembly checkpoint (SAC), blocks late cytokinesis, induces microtubule-dependent centrosome fragmentation, and increases the distance between mother and daughter centrioles in G1 cells [120].

RhoA indirectly modifies the actin cytoskeleton through ROCK1, but not ROCK2, and directly affects centriole structure and function through centriole-associated factors [121]. Similarly to ROCK, downstream LIMK inhibition also induces centrosome fragmentation [122]. Myosin II is also essential for centrosome separation and positioning during mitotic spindle assembly [123]. In addition, during interphase and mitosis, p190B Rho GAP and MgcRacGAP localize to chromosomal centromeres and provides epigenetic centromere maintenance [124, 125]. The Rho GEF ARHGEF10 regulates RhoA and controls centrosome duplication through its binding partner, the motor protein KIF3B, which colocalizes with ARHGEF10 at the centrosome [126].

Cofilin, downstream of RhoA, regulates ACD during meiosis

Meiosis occurs in oocytes that lack centrosomes [127] and possess a dynamic cytoplasmic actin network. This network is critical for spindle migration during meiosis and functions in cooperation with various actin-binding proteins [128]. Cofilin is primarily distributed around spindles. LIMK overexpression, which interferes with cofilin activation during maturation, results in the depletion of cytoplasmic F-actin. This depletion leads to defects in spindle migration and polar body extrusion [129]. Cyclase-associated proteins (CAPs) can promote actin disassembly by enhancing the actin-severing activity of cofilin. The knockdown of CAP1 leads to the accumulation of excessive actin filaments near the spindles, which impairs meiotic spindle migration and asymmetric division [130].

RhoA regulates the migration of non-tumor cells

The actin cytoskeleton endows the cell with shape, structure, and polarity and undergoes constant remodeling. Lamellipodia, filopodia, stress fibers, and focal adhesions constitute the structural framework of the actin cytoskeleton [131]. In normal epithelia, RhoA helps generate epithelial polarity and junction assembly and function [132]. The role of RhoA is complex, as the production of actin assembly drives protrusions at the front, coordinating actomyosin contractility-driven retraction at the tail to facilitate movement during cell migration in 2-D environments [133]. For example, in migrating leukocytes, cell body and tail contraction depend on actomyosin contractility and can be regulated by RhoA/ROCK [134, 135]. However, excessive activation of RhoA causes an increase in the contractile force that pulls endothelial cells and disrupts barrier function. Cofilin is required for and promotes lamellipodium extension and cell migration [136]. It has also been reported that RhoA cooperates with Rac1 and Cdc42 to induce membrane ruffles through the recruitment of mDia, implying amoeboid-like motility [137]. These findings are of particular interest with respect to explaining of epithelial cell migration.

Cell adhesion activates RhoA, leading to the activation of ROCK and mDia, organization of actomyosin bundles into stress fibers, and the formation of focal adhesion complexes composed of vinculin, paxilin, and focal adhesion kinase [51]. Tyrosine kinases are essential in signal transduction from integrins to RhoA; tyrosine phosphorylation of the focal adhesion plaque alters the assembly of actin fibers by RhoA/ROCK, which then effect the formation and aggregation of focal adhesions [138, 139]. Focal complex confined to lamellipodia can be induced by high level of Rac and low level of Rho, enlarge and elongate centripetally into typical focal contacts upon upregulation of Rho, and turn over for cells to migrate [140].

RhoA-ROCK signaling pathway promotes cancer progression

Rho GTPases, in addition to many other cellular functions, are components of the signaling network that coordinates cell proliferation with microenvironmental dynamics, and their deregulation contributes to malignant transformation and cancer. In addition to changing the adhesive repertoire, cancer cells employ developmental processes to gain migratory and invasive properties that involve dramatic reorganization of the actin cytoskeleton and concomitant formation of membrane protrusions, which are required for the invasive growth of cancer cells (Fig. 3).

RhoA promotes the migration and invasion of cancer cells by regulating EMT

During EMT, in which epithelial cells gradually dedifferentiate, polarized epithelial cells lose their adhesive properties and acquire a mesenchymal cell phenotype. As a result, the cells acquire migratory and invasive properties that allow them to penetrate the extracellular matrix (ECM) more easily [141, 142]. Cell movement in the ECM is a multistep process that requires reorganization of the actin cytoskeleton and formation of membrane protrusions. Increased intracellular pressure via RhoA-ROCK signaling leads to blebbing, thus causing amoeboid migration, while regulating tail retraction during mesenchymal migration [143, 144]. Apart from lamellipodia and filopodia, tumor cells also form lobopodia,

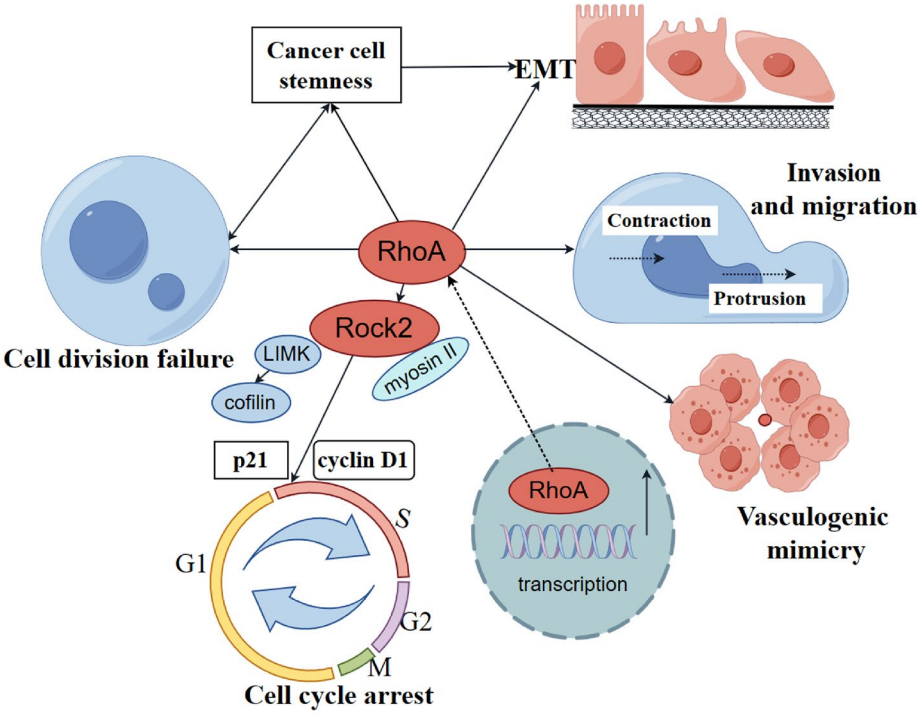

Fig. 3 RhoA is overexpressed in cancer and promotes cancer progression via RhoA/ROCK2 signaling pathway. RhoA and its downstream effectors Rock2, myosin II, LIMK, and cofilin play critical roles during G1-S phase progression by regulating the expression of cell cycle proteins p21 and cyclin D1. RhoA regulated by Rho GEFs and GAPs can recruit effectors to form a contraction ring during cell cytokinesis, and the loss of contractile forces can cause asymmetric division. RhoA-mediated actin assembly, protrusion at the front of the cell, and contraction at the body of the cell enhance motility and promote cancer cell invasion and migration. In addtion, RhoA plays an important role in epithelial-mesenchymal transition and the formation of vasculogenic mimicry network. These processes drive cancer stem cells to tumor progression

which are blunt cylindrical protrusions formed by intracellular pressure and have been discovered in the three-dimensional extracellular matrix [145].

In addition, cells also form invadopodia and podosomes, actin-rich structures that proteolytically degrade the ECM for 3D migration [146]. Proteases are released to remodel the extracellular matrix by proteolysis, such as the levels of Matrix metalloproteinase-9 correlated with invasive ability [147].

RhoA enhances the formation of vasculogenic mimicry in malignant tumors

In addition to tumor angiogenesis, aggressive tumors acquire microcirculation through vasculogenic mimicry (VM), which tumor cells form by acquiring plasticity to mimic embryonic vasculogenic networks and connect with those of the endothelium for invasion and metastasis. It is known that, RhoA/ ROCK plays an important mediated role in the process of cancer cell VM formation [148]. RhoA signaling is involved in the maintenance of actin organization, induction of migration, and tube formation in the VM [149]. Hypoxia enhances RhoA/ROCK 2 and Rac1/Pak activity, stabilizes hypoxia-inducible factor-1α (HIF-1α) protein, and induces VM formation through HIF-1α stabilization and EMT with vimentin phosphate activation [150]. In addition, axon guidance factor Sema4D activates RhoA/ROCK pathway and regulates tumor cell plasticity and migration to achieve VM in non-small cell lung cancer [151]. ROCK mediates TGF-β1-induced EMT and cancer stemness activity to participate in VM formation [152]. The traditional herbal medicines Baicalein [153] and Incarvine C [154] suppress VM by inhibiting the RhoA/ROCK signaling pathway.

RhoA signaling pathway promotes the stemness of cancer cell

Rho GTPases enhance the stemness of cancer cells by interacting with other signaling pathways, such as Notch and Wnt [155–157]. Wnt3A was found to promote superoxide generation by activating the phosphorylation of RhoA, in addition to Wnt3/RhoA/ROCK signaling pathway being involved in adhesion-mediated drug resistance of multiple myeloma [36, 158]. Loss of p130 induced expression of Wnt5A, which selectively increased RhoA transcription and drove tumorigenesis in small cell lung cancer cells [159].

YAP/TAZ are sensors of the structural and mechanical features of the cellular microenvironment. Their activation induces CSC characteristics, such as proliferation, chemoresistance, and metastasis [160]. RhoA mediates

YAP1 dephosphorylation and transport to the nucleus, inducing autophagy and promoting the migration of liver CSCs [161]. Protease-activated receptor 1 (PAR1) activation inhibits the Lats kinase associated with the Hippo-YAP pathway via Rho. Inhibition of Lats kinase results in increased nuclear localization of dephosphorylated YAP, leading to EMT [162]. Another study has shown that inhibition of the RhoA GTPase/F-actin pathway may be a useful approach for suppressing YAP/TAZ activity and limit breast CSC formation [163]. In cancer-associated fibroblasts, RhoA knockout can reduce branching and meshwork formation and may promote CSC-like properties [164].

RhoA signaling pathway is associated with the formation of polyploid giant cancer cells

Tumors are composed of non-homogeneous cell populations that exhibit varying degrees of genetic and functional heterogeneity [165]. Polyploid cancer cells (PGCCs) are a subtype of CSCs that express stem cell properties and have differentiation potential. PGCCs are large and contain single giant nuclei or multiple nuclei with significant variations in shape, chromatin pattern, and number of nucleoli; they display an ACD by budding or bursting [166–168]. PGCCs confer resistance to DNA damage and contribute to the formation of complex tumor cell karyotypes by promoting aneuploidy and chromosomal instability [169–171]. The PGCCs exhibited significant differences in the organization of actin stress fibers, including the presence of longer and thicker stress fiber bundles. Increased cytoskeletal rigidity and nuclear structure are largely regulated by the RhoA-ROCK1 signaling pathway and actin cytoskeletal dynamics, which are essential for protecting cells from injury [172]. Therefore, the regulation of the RhoA/ROCK pathway is critical to understanding how PGCCs promote tumor progression.

RhoA-ROCK2 signaling is involved in chemoradiation therapy resistance

Although most cancer cells tend to become addicted to therapy, PGCCs or other CSCs show low sensitivity, undergo transient dormancy, and can evade treatment and cause cancer relapse after emerging from their dormant state [173, 174]. For example, RhoA signaling promoted CSC phenotypes in diffuse-type gastric adenocarcinoma cells, and RhoA inhibition could reverse chemotherapy resistance both in vivo and in vitro [175]. The mechanisms of chemoresistance in cancer cells are complicated, and the main mechanisms include increased drug efflux mediated by membrane transport proteins [176–178], reduction of Bcl-2 family mediated cell apoptosis [179, 180], and reduction of topoisomerase activity, leading to decreased affinity of topoisomerase to its target cells [181]. RhoA is closely associated with multidrug resistance-associated proteins (MRPs) in the development of chemoresistance.

RhoA was mediated by GPR56 and enhanced drug resistance through upregulation of MDR1 levels [182]. RhoA knockdown increases NF-κB activation, which induces nitric oxide production and leads to tyrosine nitration of multidrug resistance protein 3 (MRP3), thereby reducing doxorubicin efflux and reversing chemoresistance [183]. Besides, inhibition of RhoA rescued the resistance to CPT-11 by inhibiting MRP1 and GSTP1 expression and promoting apoptosis [184]. Notably, RhoA exerted different effects on chemoresistance to different drugs. For example, downregulation of RhoA decreased the chemoresistance of cells to doxorubicin due to MRP1 internalization and increased doxorubicin accumulation but decreased chemoresistance to cisplatin due to decreased cisplatin influx [185, 186]. In osteosarcoma, RhoA enhances therapy resistance and suppresses apoptosis after photodynamic treatment through the Hippo/YAP pathway [187]. Mechanically, RhoA knockdown alleviates the antagonism of HIF-1α to hypoxia-induced apoptosis [188]. There is also evidence that RhoA facilitates brain metastasis during the evolution of Osimertinib-resistance in NSCLC cells [189].

In addition, elevated ROCK2 expression is associated with chemoresistance. Inhibition of ROCK2 signaling sensitizes drug-resistant prostate cancer to enzalutamide [190] and gemcitabine [191]. In MGMT-low (O6-methylguanine-DNA methyltransferase) temozolomide (TMZ) resistant glioma cells, overactive ROCK2 increased homologous recombination repair and decreased TMZ sensitivity [192]. Both sunitinib and everolimus treatment significantly enhanced ROCK2 phosphorylation and subsequent β-catenin nuclear translocation [193]. The decreased expression of ARHGAP18 (a Rho GAP protein) leads to the increase of RhoA/ ROCK1 signaling pathway and promotes glucocorticoid resistance by anti-apoptotic of leukemia cells [194].

There are reports that implicating the RhoA/ROCK pathway in radiation resistance [195]. Radioresistance in glioma cells could be induced by activation of the DNA repair pathway and increased levels of reactive oxygen species (ROS), resulting in the enrichment of CD133+CSCs [196, 197]. On the one hand, modulation of RhoA activity sensitized cells to γ-irradiation by attenuating the DNA damage response and repair pathways [198]. In addition, activated RhoA is found in the nucleus of tumor cells after irradiation and regulates resistance by modulating survivin activity [199, 200]. In contrast, RhoA is a downstream target of heterogeneous nuclear ribonucleoprotein C1/C2, and RhoA inhibition can hinder the activity of cancer-associated fibroblasts and weaken the radiation resistance of pancreatic tumors

[201]. ROCK inhibitor reduced survival and DNA repair capacity in wild-type p53 cells by inhibiting NHEJ and NER pathways with reduction of γH2AX foci and accumulation of strand breaks [202].

Small molecular inhibitors targeting RhoA-ROCK2 signaling

The implications of Rho GTPases and their upstream regulators or downstream effectors in the transformation, migration, invasion, and tumorigenesis of various CSCs highlight the potential of Rho GTPase targeting in cancer therapy [203]. First, targeting RhoA inhibitors such as Botulinum C3 exoenzyme, Grincamycin B, and Riboprine (N6-isopentenyladenosine) inhibits RhoA activity and attenuates stemness, inhibits the VM network formation, and suppresses tumor growth and invasion [204, 205]. Inhibitors of the RhoA/ROCK2 pathway shown in Table 1. The RhoA inhibitor Rhosin, which specifically binds to RhoA, was the first RhoA subfamily specific inhibitor developed to target GEF activation, and has been used in several cancer cells [139, 206, 218]. CCG-1423 is an inhibitor of RhoA/C that disrupts the transcriptional response of the Rho pathway [207]. CCG-100,602 and CCG-203,971 are new second-generation RhoA inhibitors with lower toxicity and higher selectivity and potency than those of CCG-1423 [208]. JK-136 and JK-139, two of the anti-RhoA hydrazide derivatives, were found to inhibit gastric cancer in mice [209].

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ARHGEF12, also known as LARG, regulates RhoA activity and could regulate cell morphology and invasion [219], as well as the mechanical response to integrins, mesenchymal stem cell stemness [210, 220] Y16, a small-molecule inhibitor of ARHGEF12, has been reported to inhibit RhoA activity and suppress sphere formation in breast cancer cells [218, 221]. The activity of Rho GEF Vav was important for the development of head and neck squamous cell carcinoma and showed effective inhibition in vivo [222, 223].

Post-translational modifications of RhoA may be promising cellular targets for anticancer therapy. Lovastatin suppressed EGF-induced thyroid cancer cell invasiveness by reducing Rho geranylgeranylation, which in turn suppressed membrane translocation and the subsequent suppression of Rho/ROCK and FAK/paxillin signaling [224]. PTX-100 is an inhibitor of geranylgeranyl transferase-1 (GGT-1), which mediates RhoA prenylation. It has shown significant antitumor activity in mouse models

Table 1 Inhibitors of the RhoA/ROCK2 pathway

Main target	Inhibitors	Functions
RhoA	Rhosin	Inhibit cancer cell migration and invasion [206]
Rho	CCG-1423, CCG-100,602, CCG-203,971	Inhibit cancer cell invasion and repress fibrogenesis [207, 208].
RhoA	JK-136, JK-139	Inhibit cancer activities in vivo [209].
RhoA	Grincamycin B	Suppresses the growth and invasion of glioblastoma cells; targets glioblastoma stem cells; and attenuates the formation of tumor spheres [205].
RhoA	Riboprine	Inhibits the formation of vasculogenic mimicry network and suppresses cell migration and invasion [149].
ARHGEF12	Y-16	Regulates RhoA activity and reduces cell invasion; promotes mesenchymal stem cell stemness; and enhance differentiation-related gene expression [210].
ROCK2	Fasudil	Sensitizes CSCs to chemotherapy and radiation response and suppresses the growth and tumorigenicity of chemo-resistant osteosarcoma cells [211].
ROCK	Y-27,632	Sensitizes CSCs to chemotherapy and radiation response and reduces contractility and collagen degradation capacity [212].
ROCK	PT262	Induces cytoskeleton remodeling and inhibits migration [213].
ROCK	RKI-1447	Inhibits migration, invasion, and anchorage-independent tumor growth [214].
ROCK	AT13148	Reduces tumor growth and blocks cancer cell invasion [215].
ROCK	Ripasudil	Associates with other agents to reduce tumor burden and prevent metastasis [216].
ROCK	HSD1590	Inhibits the migration of breast cancer cell [217].

of breast cancer and is well tolerated. Patients in their advanced stages of disease are currently being recruited for a phase 1 clinical trial (NCT03900442) [225, 226].

Subsequently, common ROCK chemical inhibitors include fasudil (HA-1077) and Y-27,632, which can decrease tumor growth, invasion, and metastasis [211, 227–229] and sensitize CSCs to chemotherapy and radiation [230]. Y-27,632 can reduce the contractility and collagen degradation capacity of both CSCs and non-CSCs by blocking microtubule and F-actin assembly [212]. In addition, Y-27,632 activated dormant breast cancer cells and disrupted cell junctions, thereby promoting cell proliferation, migration, and invasion [231]. Exposure of circulating breast cancer cells to Y-27,632 destabilizes the actin cortex and increases the formation of microtubules, which are microtubule-based structures that enhance their ability to reattach to the vasculature [232]. However, interestingly, transient and continuous supplementation of non-toxic concentrations of Y-27,632 and fasudil inhibits apoptosis, enhances the ability of cells to form spheres, and increases stem cell marker expression in glioblastoma stem cells [233]; Y-27,632 primes the transition of $CD44^{-/low}$ cells to generate $CD44^{high}$ cells, helping to maintain a $CD44^{high}$ fraction and tumorigenic diversity in colon cancer [234]. Other selective inhibitors of ROCK include PT262, RKI-1447, CCT129253, and AT13148; however, their evaluation as anti-metastatic agents have been restricted to solid tumor cells, such as breast cancer and melanoma [49, 213–215, 235]. Photodynamic therapy combined with a ROCK inhibitor (ripasudil) and an anti-PD-L1 antibody could reduce uveal melanoma and prevent metastasis [216]. Furthermore, HSD1590, a new compound inhibited the migration of breast cancer cell [217].

Conclusion

In summary, the tumorigenic potential of Rho GTPases has led to continuous investigation of their functions. Many hallmarks of cancer, including unlimited replicative potential, evasion of apoptosis, tissue invasion, and metastasis, may be related to abnormal cytoskeletal or matrix mechanics. Undoubtedly, the RhoA/ROCK2 signaling pathway regulates cancer stemness in response to chemotherapy and radiation therapy and is involved in aneuploidy formation through ACD. Most RhoA/ROCK inhibitors remain in laboratory use; nevertheless, their pharmaceutical development and research continue to hold promise. These inhibitors show promise as emerging targets to combat cancer progression and suppress tumor stemness.

Abbreviations

Rho GEFs	Rho guanine nucleotide exchange factors
Rho GAPs	Rho GTPase-activating proteins
Rho GDIs	Rho GDP-dissociation inhibitor
ROCK	Rho-associated coiled-coil-containing protein kinase
MLCK	myosin light chain kinase
LIMK	LIM kinase
PKA	protein kinase A
MRLC	myosin regulatory light chain
EMT	epithelial-mesenchymal transition
ECM	extracellular matrix
VM	vasculogenic mimicry
ACD	asymmetric cell division
MTOC	microtubule organizing center
SAC	spindle assembly checkpoint
PCM	pericentriolar material
PGCCs	Polyploid giant cancer cells
CSC	cancer stem cell
MRPs	resistance-associated proteins
TMZ	temozolomide

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Author contributions

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This review does not contain any studies with human or animal subjects performed by any authors.

Consent for publication

All the authors have approved the manuscript and agree with submission.

Competing interests

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