

Review Article

RhoA Cytoskeletal Pathway to Transplantation

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Abstract

RhoA is a small GTPase, which upon activation by various extracellular, transmembrane and intracellular molecules regulates, via its downstream effectors, a variety of cell structures and functions including actin cytoskeleton organization, cell motility, vesicular trafficking, cell division and tissue repair. Because of its multifunctionality the RhoA pathway is pivotal for proper functioning of the immune system and growing evidence indicate that the modulation of or interference with RhoA pathway may represent a novel approaches for successful immunosuppression and anti-rejection therapies.

Keywords

- RhoA
- Actin
- Cytoskeleton
- Antigen presentation
- Transplantation

ABBREVIATIONS

GPCR: G Protein Coupled Receptors; EphA: Ephrin A; LPAR: Lysophosphatidic Acid Receptor; KTN1: Kinetctin; GEF: Guanine Nucleotide Exchange Factors; PLD1: Phosphatidylcholine: Specific phospholipase; PRK1-Serine/Threonine-Protein Kinase N1; Rac1: member of Rho GTPase Family; ROCK: Rho-Associated Protein Kinase; FAK: Focal Adhesion Kinase; IS: Immunological Synapse; MTOC: Microtubule Organizing Center; APC: Antigen Presenting Cell; DC: Dendritic Cell

INTRODUCTION

RhoA is a member of Rho GTPase family (containing three subfamilies: Rho, Rac and CDC42), which belongs to the Ras superfamily of small GTP-binding proteins. These proteins occur in cell in two states: GTP-bound active and GDP-bound inactive state. Their activity status is regulated by a group of activator proteins: Guanine exchange factors (GEFs), and two groups of desactivators: GTPase activating proteins (GAPs) and Guanine Dissociation Inhibitors (GDIs), which control the exchange of GDP for GTP, increase the rate of GTP hydrolysis, and inhibit the release of GDP, respectively [1]. Higher vertebrates have three highly homologous Rho GTPases: RhoA, RhoB and RhoC, which despite their amino acid composition and structural similarity, regulate different cell functions [1]. The RhoA is a multifunctional protein that, through the action of its various downstream partners, regulates plethora of cell structures and functions such as actin cytoskeleton, intracellular transport, cell motility, cell cycle, cell proliferation, cell adhesion, transcription, oncogenic transformation and tissue repair. Growing number of studies indicate that RhoA pathway regulation of cellular cytoskeleton plays a critical role for proper functioning of immune response [2-8]. In this review we focus on the role of RhoA pathway in

governance of actin cytoskeleton-related functions in immune system and allograft rejection.

Activation, multifunctionality and molecular components of RhoA pathway

The RhoA is activated by a variety of extracellular, transmembrane and intracellular signaling molecules such as: guanosine nucleotide-binding proteins (G proteins) coupled receptors (GPCRs), hormones, growth factors and cytokines (Figure 1) [1,9-12]. Some of these compounds, such as: GPCRs, protein-tyrosine kinase Ephrin A (EphA) receptors, Lysophosphatidic acid (lipid metabolite that induces activation of mitogen-activated protein kinase and phagocytosis pathway) receptor (LPA), Insulin-Like Growth Factor (IGF) and its receptor, activate RhoA through the function of GEFs, while the other such as an integral transmembrane protein kinectin can activate RhoA directly (Figure 1) [13-18]. Upon activation, RhoA regulates, via protein serine/threonine kinase ROCK1 and a multitude of other downstream effectors, a network of cytoplasmic and nuclear structures and functions (Figure 1) [1,10,19,20]. Despite of dozens of RhoA-related papers being published every year there are two underappreciated and relatively rarely studied aspects of RhoA function and regulation. First is the ability of RhoA to influence chromatin structure and nuclear transcription through the control of polymerization status and accumulation of nuclear actin [21,22], and second – a reciprocal interaction between RhoA and Rac1 pathways [23,24]; the two facets, which add new dimensions to the already extremely complex RhoA regulation circuit (Figure 1) [12,25-27].

RhoA pathway regulation of immune cell functions

The involvement of RhoA pathway in regulation of actin polymerization and actin filament distribution and architecture

(Figure 1,2) suggests a priori that every cellular process that relies on proper organization of actin cytoskeleton will be affected by changes in RhoA pathway activity. Such actin-dependent processes vital for immune cell functions, include: cell motility and directional migration, extracellular matrix degradation, cell-cell interaction and formation of immunological synapse, phagocytosis and antigen presentation, and targeted cytotoxicity and killing (Figure 1) [5-7].

RhoA regulation of cell movement and matrix degradation

Upon activation, the immune cells have to be able to move toward their target. Although the amoeboid movement characteristic for lymphocytes and neutrophils, and the fibroblast type movement characteristic for macrophages differ in their velocity (amoeboid movement is about 10 times faster than fibroblast movement) both require polarization of the cell and formation of a leading (protruding) and a trailing (lagging/uropod) edge (Figure 1). The leading edge forms a lamellipodium, which contains a mesh of actin filaments and propels cell forward [8]. At the same time the adhesion of cell membrane to the substratum through focal adhesion complexes interconnected by actin/myosin stress fibers retracts the trailing edge and results in the net forward movement of the cell [8,28]. Studies on T cell lines Jurkat, HSB-2, and Peer T cell lines, human Tumor-infiltrating lymphocytes (TIL) and T lymphocytes isolated from rheumatoid

synovium of volunteer donors showed that RhoA plays a role in regulation of focal adhesions and actin stress fibers assembly [8,29], and that RhoA by being involved in T cell polarization, chemotaxis and leading edge retraction is absolutely required for T cell migration [8]. Studies on Jurkat T cells showed that RhoA (acting through its effector mDia) regulates migration and activation of T lymphocyte via regulation of actin polymerization [30]. Studies on rat cardiac allograft model system showed that a down regulation of RhoA in T cells resulted in dramatic changes in the distribution of actin and actin-binding adaptor protein, HIP-55 (hematopoietic progenitor kinase 1 [HPK1]-interacting protein of 55 kDa, also called SH3P7 and mAbp1) in these cells, which in turn, inhibited T cell infiltration into the graft [31]. Accordingly, the perioperative administration of Y-27632, which is specific inhibitor of RhoA effector molecule Rho kinase ROCK, resulted in abrogation of chronic rejection of the allograft in rat model system [32]. In dendritic cells (DCs) the RhoA pathway regulates response to the chemoattractant sphingosine 1-phosphate (S1P), which promotes DCs migration from skin and lung to draining lymph nodes *in vivo* [33]. These authors showed that Switch-associated protein 70 (SWAP-70) present in DCs interacts with active RhoA (RhoA-GTP) and Rac1 (Rac1-GTP) and regulates actin polymerization, S1P receptors and DC motility. The Swap-70 knockout bone marrow DCs fail to activate RhoA following an S1P stimulus and are unable to retract their trailing edge [33].

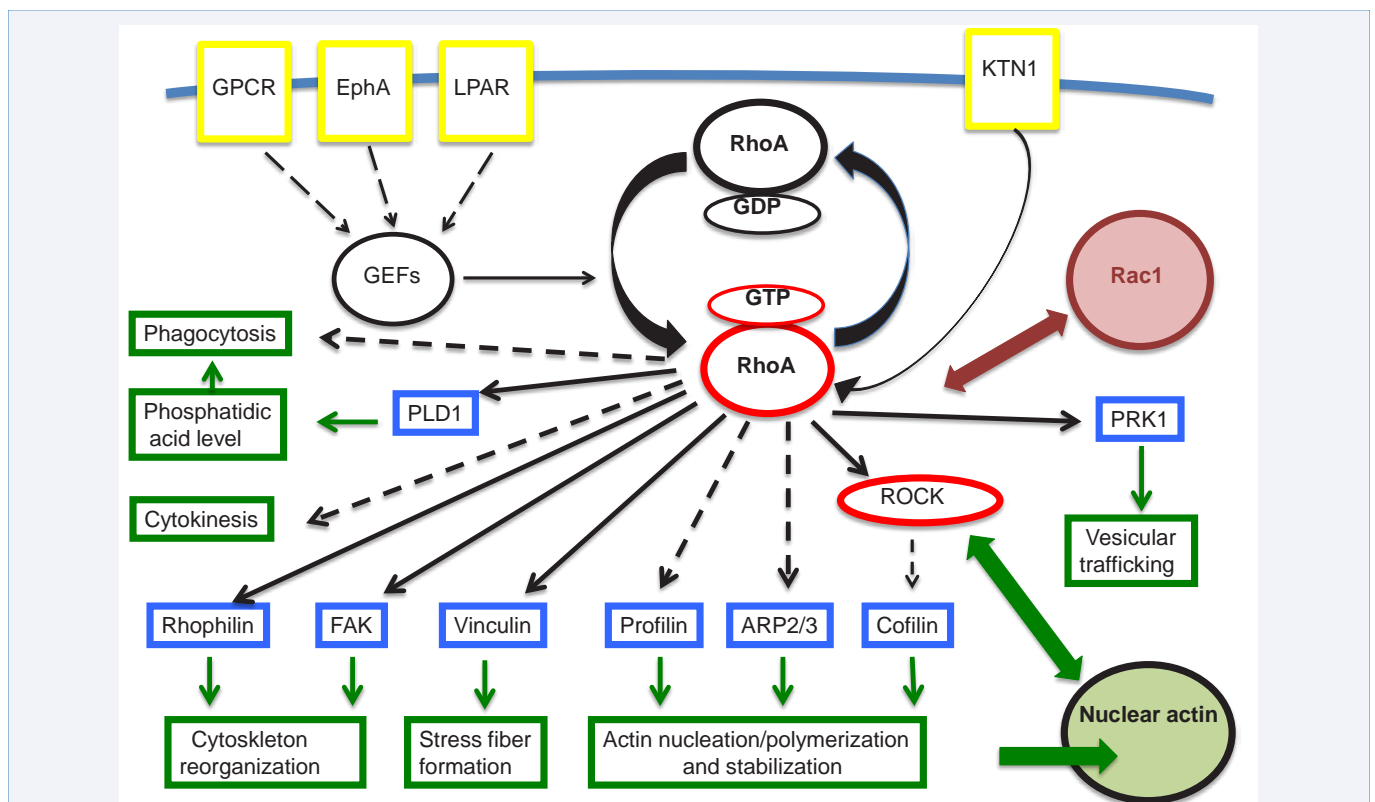


Figure 1 RhoA pathway role in actin cytoskeleton-related cell functions The RhoA switch between inactive RhoA-GDP and active RhoA-GTP form is induced by various extracellular signals and receptors (yellow boxes) acting indirectly via GEFs or is induced directly by molecules such as kinectin. Activated RhoA regulates various cellular processes (green boxes) through plethora of proteins (blue boxes) and ROCK kinase effectors. There is also a reciprocal interaction between RhoA pathway and Rac1 pathway. In addition RhoA regulates the pool of nuclear actin that in turn remodels chromatin and influences gene transcription. Dashed arrows show routes containing multiple (not shown) effector molecules.

The migration of macrophages and other myeloid leukocytes within the tissues depends not only on the formation of actin-rich leading and trailing edge, but also on the ability to degrade extracellular matrix. Proteolytic degradation of extracellular matrix depends on the function of highly specialized ventral cell membrane adhesion structures called the podosomes (Figure 2) [34]. Podosomes contain bundles of actin filaments and metalloproteinases-containing vesicles, which are used for extracellular proteolysis, and they coordinate cell movement with matrix degradation [35-37]. Formation of podosomes depends on the activity of RhoA [38] and another small GTPase, CDC42Hs and its effector Wiskott-Aldrich syndrome protein (WASp), [39]. Active Rho A (Rho GTP) is localized in podosomes (colocalizes with podosomal actin) [40] and silencing of RhoA expression or its inactivation by *Clostridium botulinum* C3 toxin ablates formation of podosomes [38,40].

RhoA regulation of TCRs and immunological synapse

Activation of T cell requires contact between the T cell receptor (TCR) and major histocompatibility complexes (MHC) expressed on antigen presenting cells (APCs) and formation of actin filament-rich interface called the immunological synapse

(IS) between the T cell and the APC [41-43]. Number of studies showed that actin cytoskeleton of IS acts as a scaffold for temporal and spatial distribution of TCRs and T cell signaling components such as Supra-Molecular Activation Clusters (SMACs) [3,41-45,47]. It has been shown that RhoA regulates (through cytoskeletal rearrangements and tetraspanin CD82) early TCR signaling and that inhibition of RhoA using dominant-negative mutants or toxins, decrease the CD82-induced T cell activation [29]. In addition, studies on transgenic mice expressing an active mutant of RhoA showed that RhoA regulates TCR-mediated responses in primary lymphocytes and that loss of function of RhoA inhibits pre-T cell differentiation and survival [48,49]. This indicates that RhoA regulates not only the functions of mature T cells but also plays a role in determining their fate. Numerous studies showed that internalization of TCRs and the assembly of SMACs - processes involved in T cell activation, depend on actin filaments and actin binding protein Hip-55 [2,5,43,48,50]. The HIP-55 knockout mice showed defective T cell proliferation, decreased cytokine production and failed to upregulate activation markers induced by TCR stimulation [51,52]. In addition, these mice had reduced immune responses such as production of antigen-specific antibodies and T cell proliferation [52]. The

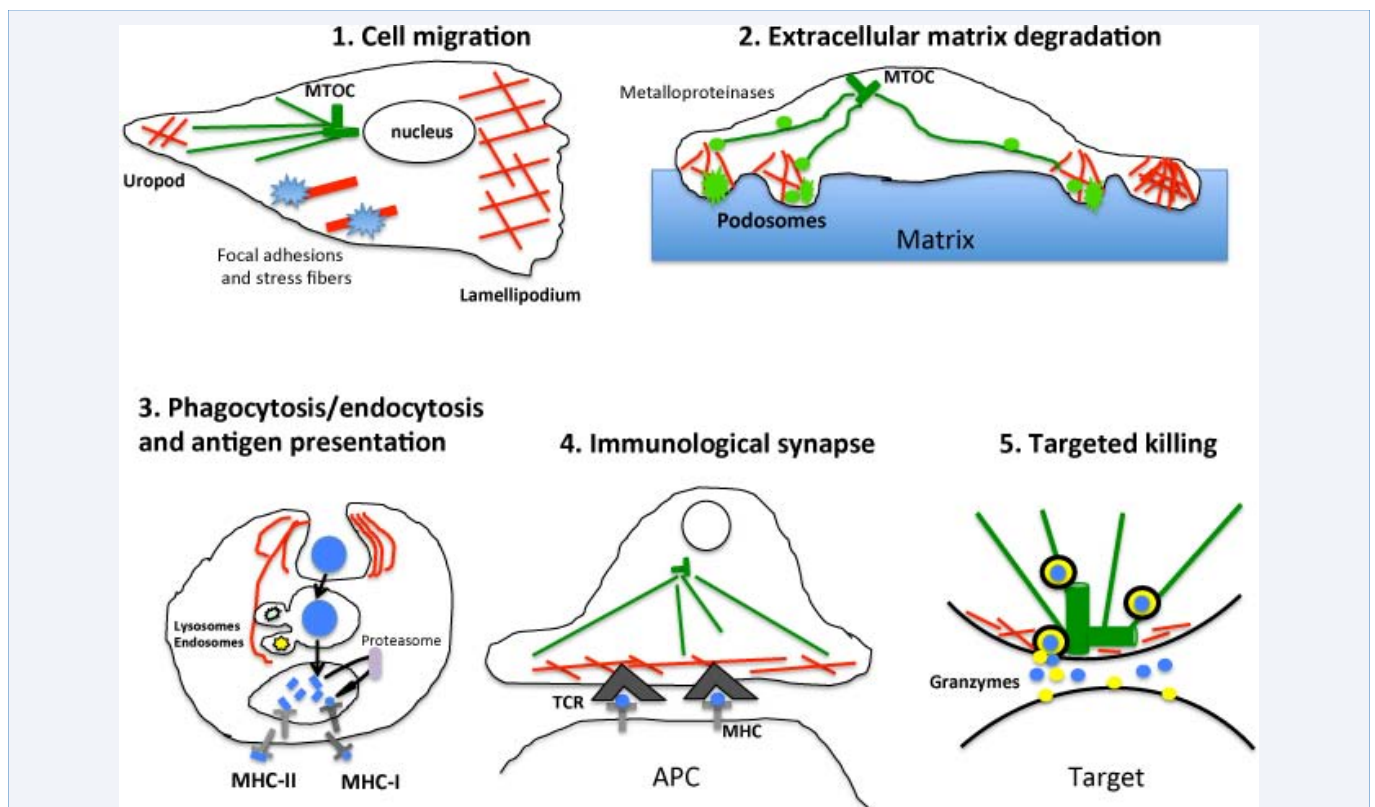


Figure 2 Actin-related cellular functions regulated by RhoA pathway. 1. In migrating cell RhoA regulates actin filament (red) polymerization in the leading and trailing edge and also focal adhesion and actin stress fibers. 2. RhoA by regulating actin filaments in podosomes influences rate of matrix degradation by metalloproteinases (green), which are delivered via microtubules emanating from the vicinity of pair of centrioles (MTOC, green). 3. During phagocytosis and endocytosis RhoA regulates through the actin filaments the formation of phagosome and its fusion with endosomes and lysosomes, which in turn is involved in processing of the antigen (blue) and antigen presentation by MHC molecules at the cell surface. 4. In immunological synapse, through the regulation of actin network, RhoA participates in TCRs and SMACs segregation, cell activation and interaction with APC. During targeted killing, reorganization of actin cytoskeleton at the immunological synapse, allows for targeted delivery of Granzyme containing granules (yellow, blue), which are released from the vesicles delivered to the synapse on the microtubules emanating from the MTOC. Part of the figure was modified from Kloc and Ghobrial [7] and Kloc et al. [6].

overexpression and RNA interference experiments showed that the HIP-55 through its interaction with protein serine/threonine kinase and small GTPase regulator HPK1 downregulates TCR expression and that HIP-55 actin-depolymerizing factor homology domains were required for this function [51]. The TCR response is regulated, *in vitro* and *in vivo*, by RhoA, and T cells expressing active RhoA show dramatic increase of TCR-induced proliferation [49]. Studies on mouse splenic T cells and Jurkat T cell line showed that the inhibition of RhoA effector molecule Rho kinase ROCK, blocks actomyosin polymerization, attenuates expression of T cell activation-related cytokines and abolishes aggregation of the T cell receptor/CD3 complexes [30]. These and other authors showed that treatment of allograft recipient with Y-27632 inhibitor prolonged the survival of fully allogeneic heart transplants in mice [30,53].

RhoA regulation of phagocytosis/endocytosis

Another actin-dependent function of immune cells is the uptake (phagocytosis/ endocytosis) and presentation of the antigen (Figure 1) [54]. The polymerization of actin filaments and proper arrangement of actin scaffold underlying cell membrane are necessary for all stages of phagocytosis: attachment of the engulfment particles/ antigens to the surface receptors, membrane excision and formation of phagosomes and phagolysosomes [55]. In all these processes actin cytoskeleton acts in unison with a membrane sculpting N-BAR protein Bin2. Live-cell imaging, siRNA interference and overexpression experiments on human and mouse B cells, macrophages and natural killer cells, showed that Bin2 is associated, via its N-BAR domain, with actin filaments underlying plasma membrane and influences rate of phagocytosis [56]. Studies of DCs showed that endocytosis is regulated by RhoA pathway via Swap 70 protein [33] and that RhoA together with Rap1 induces phagocytosis of serum-opsinized zymosan particles in mouse macrophage Raw264.7 cell line [57].

RhoA regulation of targeted killing

Actin cytoskeleton and RhoA pathway play also a role in regulation of cell-mediated killing by cytotoxic cells [58]. It has been shown that the actin cytoskeleton organization, which regulates receptor and signaling molecules at the interface (IS) between cytotoxic lymphocytes and their targets, is regulated by RhoA/p160ROCK/LIM-domain containing kinase (LIMK1) and phosphorylation of actin-binding protein cofilin [59]. This study shows that ROCK phosphorylates LIMK, which in turn phosphorylates cofilin. Cofilin phosphorylation inhibits its actin depolymerization activity, which in turn allows for stabilization of actin filaments at IS [58,60,61]. Proper arrangement of actin filaments at the killer cell/target interface is also required for the targeted release the cytolytic granules (reviewed in 6). Although the delivery of cytolytic granules toward the IS depends on microtubules, their release relies on actin and actin motor protein myosin IIA [62,63]. High-resolution imaging techniques showed that upon NK cell activation, the actin mesh underlying IS becomes remodeled- forming submicron size hypodense actin area allowing for docking and secretion of cytolytic granules [64,65]. Because these processes require extensive actin remodeling they are all controlled by components of RhoA pathway [58,60,61].

RhoA pathway inhibitors potential application in transplantation

There is increasing number of data suggesting that the regulators (modulators or inhibitors) of RhoA/cytoskeletal pathway have a potential to be used as the therapeutic agents for modulation of immune response. Studies on the immunomodulatory drugs (IMiDs) such as lenalidomide and pomalidomide showed that they regulate actin cytoskeleton in human T cells through the modulation of activity of RhoA GTPase [4]. These results led to the conclusion that a fundamental molecular mechanism by which immunomodulatory drugs influence host immune response relies on the restructuring of the cytoskeleton via modulation of GTPases activity [4].

Although there is only limited number of studies on the use of RhoA pathway inhibitor Y 27632 for inhibition of chronic rejection of cardiac allografts in rodents [30,32,53] there is an abundance of information on therapeutic function of RhoA pathway inhibitors in ischemic injury, cardiovascular diseases and pathogenesis of heart failure [listed in 4,10]. Below are the examples of presently available RhoA pathway inhibitors, which have potential to be useful as therapeutic agents in various diseases and transplantation.

Fasudil (HA-1077) -a selective RhoA/Rho kinase (ROCK) inhibitor. This is the only RhoA pathway inhibitor approved for human clinical use. Its beneficial effects were reported for treatment of cerebral vasospasms, pulmonary hypertension, memory loss and Alzheimer disease, diabetic cardiomyopathy, ischemic stroke and cardiac ischemia/reperfusion (I/R) injury [see referenes in 10]. 2. Y 27632- a selective inhibitor of p160ROCK (ROCK1) and partial inhibitor of ROCK2. Studied only in rodents and on human tissues. Prevents chronic rejection of cardiac allografts, diabetic cardiomyopathy, cardiac smooth muscle cell remodeling, atherosclerosis, I/R injury [10,30,32,53]. 3. Azaindole- 1- a ROCK kinase inhibitor [66] 4. SAR 407899- a ROCK2 kinase inhibitor, about 8 times more potent than fasudil [67]. 5. SLX-2119 (KD-025)- a selective inhibitor of ROCK2 [68], and ROCK inhibitors: 6. GSK 576371 [69], 7. GSK269962A [70] and 8. SB-772077-B 8 [71]. Inhibitors 3-8 have been mainly tested on rodents and they seem to have anti-inflammatory and -hypertension activities.

CONCLUSIONS

Although much more comprehensive studies of regulatory mechanisms pertaining to cell motility, migration and polarization as well as tissue repair and remodeling are still necessary, presented here data suggest that the RhoA pathway inhibitors, which selectively target cell actin cytoskeleton and its regulatory components, show potential to be used in pathway-targeted immunosuppression therapies.

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