Cell Biochemistry and Biophysics (2021) 79:493–496
https://doi.org/10.1007/s12013-021-00993-y

REVIEW PAPER

Lysophosphatidic Acid Regulates Rho Family of GTPases in Lungs

Kevin C. Tran[1] · Jing Zhao[1,2]

Accepted: 11 May 2021 / Published online: 10 June 2021

Abstract

The bio-active lipid, lysophosphatidic acid (LPA) interacts with various lysophosphatidic acid receptors (LPARs) to affect a variety of cellular functions, including proliferation, differentiation, survival, migration, morphogenesis and others. The Rho family of small GTPases, is well-known downstream signaling pathways activated by LPA. Among the Rho GTPases, RhoA, Rac1, and Cdc42 are best characterized and LPA-induced activation of the GTPases RhoA, Rac1, and Cdc42 influences a wide range of cellular processes and functions such as cell differentiation, contractile movements, cellular migration, or infiltration. In this review, we will briefly discuss the interplay between LPA and each of these three Rho family proteins, summarizing the main interactions between them. Our discussion will focus mainly on their interplay within lung endothelial and epithelial cells, drawing attention to how these interactions may contribute to pro-inflammatory processes.

Keywords LPA · RhoA · Cdc42 · Rac1 · Lung endothelial and epithelial

Introduction

LPA is a lysophospholipid that regulates multiple biological processes, including proliferation, cellular morphology, and endothelial permeability. LPA is produced through hydrolysis of phosphatidic acids or cleavage of lysophospholipids, the different forms of lysophosphatidic acid bind with six well-studied LPA receptors (LPARs), transmembrane proteins whose activation leads to different cellular responses [1, 2]. These variable responses are dependent on which downstream proteins are recruited as well as their interactions. LPA levels are elevated in cases of pulmonary fibrosis, hinting at its role in promoting inflammatory effects within multiple cell types [3]. Outside of its inflammatory effects, the different signaling pathways induced by LPA play roles in development, cellular proliferation, differentiation, as well as apoptosis.

✉ Jing Zhao
jing.zhao@osumc.edu

[1] Dorothy M. Davis Heart & Lung Research Institute, The Ohio State University, Columbus, OH, USA

[2] Department of Physiology and Cell Biology, The Ohio State University, Columbus, OH, USA

The Rho family of proteins are a branch of the Ras superfamily of small GTPases. They are critical participants in signaling cascades and pathways, hydrolyzing GTP to activate or regulate other proteins such as kinases [4, 5]. Rho GTPases are regulated by the enzymatic activities of GTPase-activating proteins, guanine nucleotide exchange factors (GEFs) and GDP dissociation inhibitors [6–8]. These GTPases help regulate cytoskeletal organization, cellular migration, proliferation, and apoptosis [4, 5]. As a signaling molecule, LPA can regulate these GTPases to elicit specific cellular responses (Fig. 1). In this review, we briefly examine the interactions and effects between LPA and RhoA, Rac1, Cdc42 from the Rho family of GTPases, characterizing their behaviors mainly within the context of endothelial and epithelial cells in lung tissue (Fig. 2).

LPA Regulates RhoA Activation

RhoA, along with Rac1 and Cdc42, are regulators for the actin cytoskeleton within endothelial and epithelial cells, affecting a variety of cellular functions [9]. RhoA is known to regulate formation of contractile stress fibers in non-muscle cells, which enable cellular migration and movement [10, 11]. RhoA also is involved in regulation of angiogenesis and extension wound repair driven by LPA, being essential for such within in vitro endothelial cells but partly optional for in vivo cells [12]. When activated by

LPA/LPARs, RhoA and Rho kinase may contribute to dysfunction of the endothelial barrier [13, 14]. Activation of RhoA mediates the phosphorylation of myosin light chains within actin-myosin bundles found within non-muscle cells, leading to contractile movements at cell peripheries. These contractions permit the formation of gaps between cells, such as the endothelial cells lining blood vessels [15]. The permeability of the endothelial barrier is temporarily increased, but this induced hyperpermeability is less effective than the degree of hyperpermeability induced by thrombin [13]. Inducible hyperpermeability plays a role in inflammation, permitting infiltration by other cell types through the barrier.

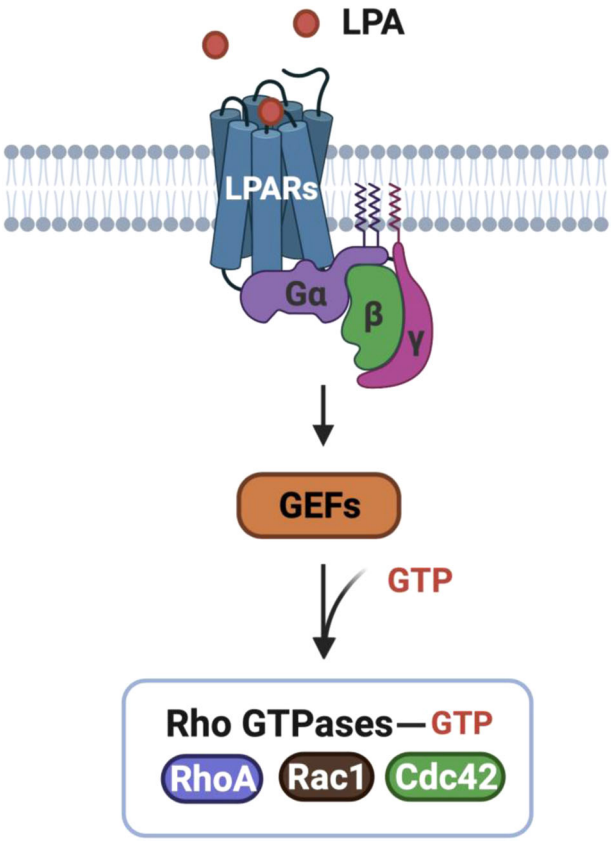

Fig. 1 Rho GTPases activiation by lysophosphatidic acid (LPA). Upon LPA binding, LPARs undergo conformational changes and induce activation of heterotrimeric G proteins. GEFs serving as the direct downstream effectors of heterotrimeric G proteins acivates Rho GTPase by simulating formation of the GTP-bound state. LPARs, receptors for LPA; G protein heterotrimeric complex: α or βγ subunits. Scheme created using Biorender.com

RhoA is also involved in the assembly and development of the apical cell surface in multiciliated epithelial cells, such as those lining the proximal and larger airways [16]. Further down the airway, the alveolar epithelium consists of type II and type I cells, where type II produces surfactant and type I composes gas exchange surfaces [17]. Breathing is a cyclic motion that can trigger stretch-induced cell signaling, which can result in a variety of effects such as increased ion channel expression or activation of substances like transforming growth factor-β (TGF-β) [18, 19]. As a result of this signaling, type II cells are able to transition into type I cells, a process mediated by the Rho protein family [20]. A previous study using Normal Human Bronchial Epithelial cells (NHBE) found that LPA induces TGF-β activation [3]. Upon LPA binding to receptor LPA2, a signaling cascade is initiated through RhoA and Rho kinase, affecting the αvβ6 integrin and subsequently activating TGF-β. This activation was found to be dependent on LPA concentration. As TGF-β is an important mediator of lung injury and fibrosis [21–23], LPA and RhoA can play profibrotic roles.

LPA Regulates Rac1 Activation

Rac1 shares similar functions to RhoA, such as reorganizing the actin cytoskeleton, cell migration and transformation [9]. Activation of Rac1 triggers the formation of a lamellipodia, a projection of actin which provides a means of cell motility [24]. Studies with Rac1 knockout mice demonstrated reduced and defective migration of neutrophils across membranes, such as the endothelial barrier [25]. In comparison to wild-type mice, loss of Rac1 in neutrophils

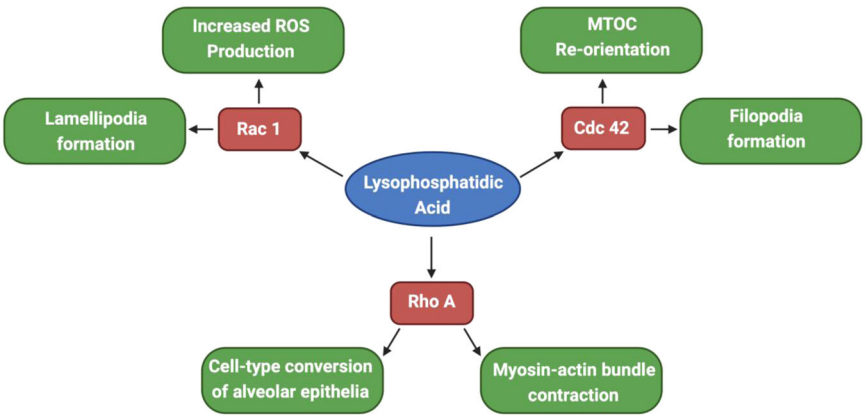

Fig. 2 LPA can induce a variety of processes or functions through interactions with one of the 3 GTPases from the Rho Family, as outlined in this graphic. These proteins' functions are not mutually exclusive or isolated from one another, some working together to achieve overall cell motility or migration. Scheme created using Biorender.com

exhibited a reduced number of neutrophils infiltrating lung interstitial and alveolar spaces. In addition, there was abnormal localization of other substances such as RhoA or improper assembly or function of actin filament structures [25]. It is also interesting to note that LPA can increased oxidative stress through increased reactive oxygen species production in pulmonary microvascular endothelial cells [26]. This study demonstrated that this effect was achieved mainly though increased activation of NOX2, which coincided with Rac1 activation and translocation to the plasma membrane. Inactivation of the LPA receptor via inhibition or knockdown diminished NOX2 activity, demonstrating another interaction between LPA and Rac1 [26]. In another study utilizing mouse lung epithelial cells, decreased expression of Rac1 through overexpression of FBXL19 lead to reduced epithelial cell migration following stimulation by LPA [27].

LPA Regulates Cdc42 Activation

Activation of Cdc42 triggers filopodia extensions, cytoplasmic projections along the leading edge of the lamellipodia structure formed from Rac activation [28]. A previous study demonstrated how upon stimulation via LPA treatment, Cdc42, alongside Rac, were essential to endothelial cell spreading [29]. Knockout of either GTPase resulted in rounded cells which did not spread effectively following LPA stimulus, a result which was similarly achieved by inhibiting LPA itself through use of pertussis toxin. Another study found that outside of changes in actin distribution or structures, Cdc42 also mediated the reorientation of microtubule-organization centers (MTOC) in fibroblasts and endothelial cells following stimulation by LPA [30]. It was seen that Cdc42, but not Rho or Rac, was necessary and sufficient for MTOC reorientation, a necessity to permit the formation of stable microtubules along the leading edge of a migrating cell. The effects of LPA on Cdc42 are important to cellular migration.

Conclusion

Lysophosphatidic acid is an important bioactive molecule which contributes to many important cellular processes. Through its interactions with RhoA, Rac1, and Cdc42, we see that LPA induces mechanisms and pathways which lead to the assembly of structures necessary to cellular migration and mobility. Some effects of LPA contribute to or permit the infiltration by some cells into other tissues, as seen with endothelial barrier permeability and neutrophils. The effects and processes in which LPA partake in as a signaling molecule are important components to inflammatory responses, demonstrating one of the many important roles that lysophosphatidic acid plays.

Acknowledgements This work was supported by grants from National Institutes of Health (R01 GM115389, R01HL151513 to J.Z.).

Compliance with Ethical Standards

Conflict of Interest The authors declare no competing interests.

References

1. Sheng, X., Yung, Y. C., Chen, A. & Chun, J. (2015). Lysophosphatidic acid signalling in development. *Development*, *142*, 1390–1395.
2. Lin, M. E., Herr, D. R. & Chun, J. (2010). Lysophosphatidic acid (LPA) receptors: signaling properties and disease relevance. *Prostaglandins & Other Lipid Mediators*, *91*, 130–138.
3. Xu, M. Y., Porte, J., Knox, A. J., Weinreb, P. H., Maher, T. M. & Violette, S. M. et al. (2009). Lysophosphatidic acid induces alphavbeta6 integrin-mediated TGF-beta activation via the LPA2 receptor and the small G protein G alpha(q). *The American Journal of Pathology*, *174*, 1264–1279.
4. Phuyal, S. & Farhan, H. (2019). Multifaceted Rho GTPase signaling at the endomembranes. *Frontiers in Cell and Developmental Biology*, *7*, 127.
5. Sadok, A. & Marshall, C. J. (2014). Rho GTPases: masters of cell migration. *Small GTPases*, *5*, e29710.
6. Dovas, A. & Couchman, J. R. (2005). RhoGDI: multiple functions in the regulation of Rho family GTPase activities. *Biochemical Journal*, *390*, 1–9.
7. Moon, S. Y. & Zheng, Y. (2003). Rho GTPase-activating proteins in cell regulation. *Trends in Cell Biology*, *13*, 13–22.
8. Rossman, K. L., Der, C. J. & Sondek, J. (2005). GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. *Nature Reviews Molecular Cell Biology*, *6*, 167–180.
9. Sit, S. T. & Manser, E. (2011). Rho GTPases and their role in organizing the actin cytoskeleton. *Journal of Cell Science*, *124*, 679–683.
10. Tojkander, S., Gateva, G. & Lappalainen, P. (2012). Actin stress fibers-assembly, dynamics and biological roles. *Journal of Cell Science*, *125*, 1855–1864.
11. Burridge, K. & Guilluy, C. (2016). Focal adhesions, stress fibers and mechanical tension. *Experimental Cell Research*, *343*, 14–20.
12. Zahra, F. T., Sajib, M. S., Ichiyama, Y., Akwii, R. G., Tullar, P. E. & Cobos, C. et al. (2019). Endothelial RhoA GTPase is essential for in vitro endothelial functions but dispensable for physiological in vivo angiogenesis. *Scientific Reports*, *9*, 11666
13. van Nieuw Amerongen, G. P., Vermeer, M. A. & van Hinsbergh, V. W. (2000). Role of RhoA and Rho kinase in lysophosphatidic acid-induced endothelial barrier dysfunction. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *20*, E127–133.
14. van Nieuw Amerongen, G. P., van Delft, S., Vermeer, M. A., Collard, J. G. & van Hinsbergh, V. W. (2000). Activation of RhoA by thrombin in endothelial hyperpermeability: role of Rho kinase and protein tyrosine kinases. *Circulation Research*, *87*, 335–340.
15. Escribano, J., Chen, M. B., Moeendarbary, E., Cao, X., Shenoy, V. & Garcia-Aznar, J. M. et al. (2019). Balance of mechanical forces drives endothelial gap formation and may facilitate cancer and immune-cell extravasation. *PLOS Computational Biology*, *15*, e1006395.

16. Sedzinski, J., Hannezo, E., Tu, F., Biro, M. & Wallingford, J. B. (2017). Correction: RhoA regulates actin network dynamics during apical surface emergence in multiciliated epithelial cells. *Journal of Cell Science*, *130*, 1017.
17. Ward, H. E. & Nicholas, T. E. (1984). Alveolar type I and type II cells. *Australian and New Zealand Journal of Medicine*, *14*, 731–734.
18. Froese, A. R., Shimbori, C., Bellaye, P. S., Inman, M., Obex, S. & Fatima, S. et al. (2016). Stretch-induced activation of transforming growth factor-β1 in pulmonary fibrosis. *American Journal of Respiratory and Critical Care Medicine*, *194*, 84–96.
19. Mustafa, S. B., Isaac, J., Seidner, S. R., Dixon, P. S., Henson, B. M. & DiGeronimo, R. J. (2014). Mechanical stretch induces lung α-epithelial Na(+) channel expression. *Experimental Lung Research*, *40*, 380–391.
20. Foster, C. D., Varghese, L. S., Gonzales, L. W., Margulies, S. S. & Guttentag, S. H. (2010). The Rho pathway mediates transition to an alveolar type I cell phenotype during static stretch of alveolar type II cells. *Pediatric Research*, *67*, 585–590.
21. Bartram, U. & Speer, C. P. (2004). The role of transforming growth factor beta in lung development and disease. *Chest*, *125*, 754–765.
22. Fernandez, I. E. & Eickelberg, O. (2012). The impact of TGF-β on lung fibrosis: from targeting to biomarkers. *Proceedings of the American Thoracic Society*, *9*, 111–116.
23. Pittet, J. F., Griffiths, M. J., Geiser, T., Kaminski, N., Dalton, S. L. & Huang, X. et al. (2001). TGF-beta is a critical mediator of acute lung injury. *Journal of Clinical Investigation*, *107*, 1537–1544.
24. Waterman-Storer, C. M. & Salmon, E. (1999). Positive feedback interactions between microtubule and actin dynamics during cell motility. *Current Opinion in Cell Biology*, *11*, 61–67.
25. Filippi, M. D., Szczur, K., Harris, C. E. & Berclaz, P. Y. (2007). Rho GTPase Rac1 is critical for neutrophil migration into the lung. *Blood*, *109*, 1257–1264.
26. Vázquez-Medina, J. P., Dodia, C., Weng, L., Mesaros, C., Blair, I. A. & Feinstein, S. I. et al. (2016). The phospholipase A2 activity of peroxiredoxin 6 modulates NADPH oxidase 2 activation via lysophosphatidic acid receptor signaling in the pulmonary endothelium and alveolar macrophages. *The FASEB Journal*, *30*, 2885–2898.
27. Zhao, J., Mialki, R. K., Wei, J., Coon, T. A., Zou, C. & Chen, B. B. et al. (2013). SCF E3 ligase F-box protein complex SCF (FBXL19) regulates cell migration by mediating Rac1 ubiquitination and degradation. *The FASEB Journal*, *27*, 2611–2619.
28. Krugmann, S., Jordens, I., Gevaert, K., Driessens, M., Vandekerckhove, J. & Hall, A. (2001). Cdc42 induces filopodia by promoting the formation of an IRSp53:Mena complex. *Current Biology*, *11*, 1645–1655.
29. Ueda, H., Morishita, R., Yamauchi, J., Itoh, H., Kato, K. & Asano, T. (2001). Regulation of Rac and Cdc42 pathways by G(i) during lysophosphatidic acid-induced cell spreading. *Journal of Biological Chemistry*, *276*, 6846–6852.
30. Palazzo, A. F., Joseph, H. L., Chen, Y. J., Dujardin, D. L., Alberts, A. S. & Pfister, K. K. et al. (2001). Cdc42, dynein, and dynactin regulate MTOC reorientation independent of Rho-regulated microtubule stabilization. *Current Biology*, *11*, 1536–1541.