Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Ripasudil alleviated the inflammation of RPE cells by targeting the miR-136-5p/ROCK/NLRP3 pathway

Zhao Gao
Shanxi Eye Hospital
Qiang Li
Shanxi Bethune Hospital
Yunda Zhang
Shanxi Eye Hospital
Haiyan Li
Shanxi Eye Hospital
Xiaohong Gao
Shanxi Eye Hospital
Zhigang Yuan (yzgsxeye@sina.com)
Shanxi Eye Hospital

Research article

Keywords: Ripasudil, ROCK1, ROCK2, miR-136-5p, RPE cells, NLRP3

Posted Date: January 13th, 2020

DOI: https://doi.org/10.21203/rs.2.20697/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Version of Record: A version of this preprint was published at BMC Ophthalmology on April 6th, 2020. See the published version at https://doi.org/10.1186/s12886-020-01400-5.

Abstract

Introduction

Inflammation of RPE cells lead to different kinds of eye diseases and affect the normal function of the retina. Furthermore, higher levels of ROCK1 and ROCK2 induced injury of endothelial cells and many inflammatory diseases of the eyes. Ripasudil which was used for the treatment of glaucoma was one kind of the inhibitor of ROCK1 and ROCK2. But whether the ripasudil could relieved the LPS induced inflammation damage of RPE cells was not clear.

Material and methods

We used the LPS to stimulate ARPE-19 cells which is the RPE cell line. After that, we detected the levels of ROCK1 and ROCK2 by western-blotting assay after the stimulation of LPS and treatment of ripasudil. Then luciferase reporter assays were used to confirm the targeted effect of miR-136-5p on ROCK1 and ROCK2. At last, the levels of NLRP3, ASC, caspase1, IL-1β and IL-18 were detected with the western-blotting after the knockdown of miR-136-5p.

Results

The levels of ROCK1, ROCK2 and miR-136-5p in ARPE-19 cells was promoted after the stimulation of LPS. After the treatment of ripasudil, the ROCK1, ROCK2 and miR-136-5p was suppressed. The miR-136-5p targeted and inhibited the expression of ROCK1 and ROCK2. Inflammation related proteins NLRP3, ASC, caspase1, IL-1β and IL-18 was inhibited after the treatment of ripasudil. However, the expression of these proteins was rescued after the knockdown of the miR-136-5p.

Conclusion

Ripasudil relieved the inflammatory injury of RPE cells by upregulating miR-136-5p and therefore inhibiting the expression of ROCK1, ROCK2, NLRP3, ASC, caspase1, IL-1β and IL-18.

Introduction

The monolayer pigment cells between the choroid and retina was called the retinal pigment epithelium (RPE). Inflammatory response of RPE and apoptosis of RPE cells were the key factors in the pathogenesis of many retinal degenerative diseases [1]. The inflammation of the RPE cells could also lead to the occurrence and development of age-related macular degeneration (AMD) and impact the normal physiological function of retina [2]. Rho-associated coiled-coil containing protein kinase (ROCK) which has two isomers ROCK1 and ROCK2 is a serine-threonine kinase which exist inside the cells. During the pathological process, excessive activation of rock-related proteins leads to the damage of vascular endothelial which may induce angina pectoris, ischemic stroke and other cardiovascular and cerebrovascular diseases [3]. The migration of endothelial cells was also regulated with the ROCK pathway [3–5]. ROCK1 and ROCK2 were expressed in many organs including the retina. The excessive

expression of the ROCK1 and ROCK2 caused many diseased in the eye, such as diabetic retinopathy and glaucoma [6, 7].

The ripasudil was the inhibitor of ROCK pathway. Therefore, ripasudil was used as a drug for the treatment of glaucoma and some other eye diseases [6]. Application of the ripasudil could reduce the IOP of eyes [8]. The ripasudil could also reduce the apoptosis of corneal endothelial cell [9]. At the same time, ripasudil could relieved the LPS induced vascular endothelial injury by targeting the ROCK2/eNOS signaling pathway [10]. Furthermore, The studies have confirmed that lncRNA SNHG14 promoted the occurrence and development of inflammation by inhibiting the intracellular content of miR-136-5p which could lead to the upregulation of the ROCK1 [11]. However, there is also research revealed that the overexpression of miR-136-5p could promoted the expression of NF-κB therefore aggravate the damage of the inflammation response [12]. However, if the ripasudil could affect the inflammation and apoptosis of RPE cells by targeting the miR-136-5p/ROCK axis was unclear.

In this study, we used the LPS induced RPE cells was used to simulate the inflammation of retina pigment epithelium. Next, the luciferase reporter assay was used to detect the targeting effect of miR-136-5p on the ROCK1 and ROCK2, meanwhile the levels of ROCK1 and ROCK2 were determined after the treatment of ripasudil and LPS. At last, levels of inflammatory factors and apoptosis of the RPE cells which was stably knockdown of miR-136-5p were determined after the treatment of ripasudil or the stimulation of LPS. The results from all these experiments will clarify the efficacy of miR-136-5p on the inflammatory response and apoptosis of RPE cells which was induced by the ripasudil.

Material And Methods

Cell culture. The RPE cell line ARPE-19 was obtained from the ATCC (Manassas, VA). And these cells were cultured in the RPMI-1640 medium (Gibco, Gaithersburg, MD, USA) which was added with 10% fetal bovine serum (Gibco, USA). These cells were placed in the 37℃ humid atmosphere with 5% CO_2. The LPS and ripasudil was added in the culture medium to simulate and treat the RPE cells. The inhibitor of the miR-136-5p (Ribbio, Guangzhou China) was transfected with the lipo-2000. All the operations of the experiment were carried out according to the instructions.

Luciferase reporter assay. 1×10^5 cells was plated into the 24 well plates. After the attachment of these cells, 0.1 μg reporter vector with 3'UTR-ROCK1 or mutant-3'UTR-ROCK1 (Genechem, China), 0.02 μg Renilla luciferase vector (Genechem, China) and the miR-136-5p inhibitor or the NC was contransfected into these cells. The targeting effect of miR-136-5p on the ROCK2 was detected by the same way. After 48 hours, the luciferase activity was measured. All the operations in this experiment followed the manufacturer's recommendations.

Western-blotting. Proteins of these cells were lysed and collected with the RIPA (Beyotime, China). Next, the concentration of these proteins was determined with the BCA (Beyotime, China) method. Then these proteins were separated with the 10% SDS-PAGE gel (Beyotime, China). Next, the proteins were transferred

to the PVDF membrane (Millipore, USA). The PVDF membrane was blocked with the 5% skim milk powder for 1.5 h. Then the membranes were incubated with the primary antibody at 4℃ overnight. The primary antibodies were ROCK1 (#4035, CST), ROCK2 (#9029, CST), Bcl-2 (#4223, CST), Bax (#5023, CST), Cleaved-caspase3 (#9664, CST), Cleaved-caspase9 (#20750, CST), caspase-3(#14220, CST), caspase-9 (#9502, CST), NLRP-3 (#15101, CST), ASC (#13833, CST), Caspase-1 (#24232, CST), IL-1β (#12703, CST), IL-18 (Abcam, ab191152) and p-65 NF-κB (Abcam, ab16502). In the second day, the membranes were washed with the PBST three times. Next, the membranes were incubated with the second antibody (Abcam, ab205718). After that the membranes were washed three times with the PBST. Final, the membranes were exposed and the band was observed to clarify the changes of expression of these proteins.

RT-PCR. Total RNA was extracted from the cells by the trizol methods. After that, the mRNA was reverse transcribed into cDNA by the RT reagent Kit (TakaRa, Japan). The RT-PCR was performed with the SYBR Green method. The amplification process was performed with the ABI 7500 system (Applied Biosystems, USA). The relative expression of the target genes was calculated by the $2^{-\Delta\Delta Ct}$ method. The following primers were used in this project: miR-136-5p forward primer 5'-ACACTCCAGCTGGGACTCCATTTGTTTT-3'; reverse primer 5'-CCAGTGCAGGGTCCGAGGT-3'; GAPDH forward primer 5'-AATTCCATGGCACCGTCAAG-3'; reverse primer 5'-TGGACTCCACGACGTACTC-3'.

CCK-8 assay. CCK-8 assays were used to detect the cells viability after the stimulation of the LPS. The cells were plated into the 96 well plate. After that, the cells were cultured with the medium containing LPS for 24 hours. Then the CCK-8 (Dojindo, Japan) was diluted with the cultured medium and added into the 96 well plate. The 96 well plate was incubated in the incubator for two hours and final the absorbance was measured with the microplate Reader.

ELISA assay. To determine the changes of TNF-α, IL-6 and IL-1β releasing of RPE cells in response to different stimulation. The levels of TNF-α, IL-6 and IL-1β in the culture supernatant was determined by the ELISA assay. The ELISA kit was obtained from Abcam and Sigma-Aldrich: TNF-α (Abcam, ab181421), IL-6 (Abcam, ab178013), IL-1β (Sigma-Aldrich, RAB0273). All the operation of this experiment was carried out according to the instruction.

Apoptosis assay. The different groups of RPE cells were seeded into the six well plates and treated with LPS or ripasudil. After that, these cells were washed with the cold PBS for three times. Then the binding buffer (Beyotime, China) was used to suspend these cells. Next, the PI and Annexin V (Beyotime, China) were added into the binding buffer to detect the apoptosis ratios of the RPE cells. The cells were incubated with the PI and Annexin V for 40 minutes. At last, the apoptosis ratios of these cells were analyzed with the flow cytometry (Beckman, USA).

Statistical analysis. The analysis of the data in this paper was performed with the GraphPad Prism 7.0. The comparison between different groups was evaluated by the Student's t test. All the data in this research were presented as mean ± SD and all the experiments in this paper were repeated three times.

The difference between diverse groups was considered statistically significant differences when the values of P less than 0.05.

Results

Ripasudil protected the RPE cells from the LPS induced inflammatory stimulation. The LPS was used to stimulate the ARPE-19 cells. After that, the levels of ROCK1 and ROCK2 and the cell viability were determined to evaluate the damage caused by the inflammation. The results (Fig. 1A) showed that the expression of the ROCK1 and ROCK2 was enhanced after the stimulation of the LPS. And the cell viability gradually decreased with the increase LPS dose (Fig. 1B). Next, ripasudil which is the ROCK inhibitor was used to treat the ARPE-19 cells which was simulated by the LPS. As shown in Fig. 1C, the cell viability enhanced after the treatment of the ripasudil. All the results in this part indicated the ripasudil could relieve the inflammatory injury which was induced by the LPS.

Ripasudil relieved the inflammatory damage of RPE cells by targeting miR-136-5p. There was study revealed that the miR-136-5p could suppress the expression of ROCK1 [11]. In this study, we used the RT-PCR to detect the level of miR-136-5p after the stimulation of LPS and treatment of ripasudil. The results (Fig. 2A) showed that the expression of miR-136-5p was inhibited after the stimulation of LPS, however, the levels of miR-136-5p was upregulated after the treatment of ripasudil. Next, we construct the miR-136-5p inhibitor, the vector and miR-136-5p was transfected into the RPE cells. The results (Fig. 2B) from RT-PCR showed that the level of miR-136-5p was downregulated in these cells. After that, the luciferase reporter assay was performed to evaluate the targeting effect of miR-136-5p on the ROCK1 and ROCK2. To detect the targeted effect of miR-136-5p on ROCK1 and ROCK2, we established the luciferase construct (Fig. 2C) which contained the wild type or mutant of 3’ UTR of ROCK1 and ROCK2 to confirm that. As shown in the Fig. 2D, the reporter assays revealed that the inhibitor of miR-136-5p enhanced the luciferase activity, mutation of miR-136-5p binding site of ROCK1 and ROCK2 3'UTR abrogated the efficacy of miR-136-5p. After that, we determined the level of ROCK1 and ROCK2 after the application of miR-136-5p inhibitor and the treatment of ripasudil or LPS. The results (Fig. 2E) showed that the levels of ROCK1 and ROCK2 were upregulated after the stimulation of LPS, but declined sharply after the treatment of ripasudil. However, the expression of miR-136-5p was rescued in the miR-136-5p inhibitor group compared to the NC group (Fig. 2D).

Inhibition of miR-136-5p induced the expression of inflammatory factors and apoptosis of RPE cells. The aberrant upregulation of inflammation factors was the main reason for the eye diseases, such as diabetic retinopathy and glaucoma. Therefore, the ELISA was performed to detect the levels of TNF-α, IL-6 and MCP-1. The results (Fig. 3A) showed that the levels of TNF-α, IL-6 and MCP-1 increased after the stimulation of LPS but declined after the treatment of ripasudil. After the suppression of the miR-136-5p, the expression of these inflammation factors was rescued. Next, the apoptosis rates of the ARPE-19 cells was detected to explore the effects of ripasudil and miR-136-5p on these cells which were stimulated by the LPS. As shown in Fig. 3B and Fig. 3C, the ripasudil relieved the apoptosis which was induced by the LPS, whereas the inhibition of the miR-136-5p aggravated the apoptosis of ARPE-19 cells. At last, the

expression of the apoptosis related proteins was determined by the western-blotting. The results (Fig. 3D) showed that the levels of the Bcl-2 decreased while the expression of Bax, cleaved-caspase3 and cleaved-caspase9 was inhibited after the treatment of ripasudil. However, after the inhibition of miR-136-5p, the levels of Bax, cleaved-caspase3 and cleaved-caspase9 were enhanced and the expression of Bcl-2 was repressed.

Ripasudil inhibited the inflammatory response by targeting NLRP3/ASC/caspase-1 pathway. The NLRP3/ASC/pro-caspase1 was a protein complex which played a critical role in the inflammatory process [13]. The formation of this complex lead to the activation of the caspase-1, the active caspase-1 could induce the expression of IL-1β and IL-18. Therefore, the western-blotting was performed to detect these proteins. As shown in Fig. 4 the NLRP3, ASC, caspase-1, IL1β, IL-18 and NF-κB p65 was inhibited after the treatment of ripasudil. Nevertheless, the levels of these proteins partially recovered after the inhibition of the miR-136-5p.

Discussion

Inflammation was a potential cause of many eye diseases, including age-related macular degeneration (AMD), autoimmune uveitis, glaucoma and diabetic retinopathy [14]. RPE cells were invaded by various inflammatory mediators and infectious factors because of their crucial position and physiological activity [15]. The inflammation of the RPE cells could finally lead to the occurrence and development of the AMD. AMD was the leading cause for the loss of vision in older people group [16]. On the other hand, ROCK1 and ROCK2 play a critical role in regulating endothelial function. It was clear that the ROCK pathway could lead to the dysfunction of endothelium by activating the NF-κB, therefore the higher levels of ROCK1 and ROCK2 was associated with the occurrence and development of inflammation injury [17, 18]. However, ripasudil which was used as a drug for the treatment of glaucoma in Japan was also one of the inhibitors of ROCK1 and ROCK2 [19]. And there is the research revealed that the ripasudil could alleviate the LPS induced inflammation and apoptosis of pulmonary microvascular endothelial cells [10]. Furthermore, the study also showed that the ripasudil hydrochloride hydrate could relieve the inflammation and intraocular hypertension [20]. In our study, we found that the stimulation of LPS lead to the upregulation of ROCK1 and ROCK2 while the treatment of ripasudil inhibited the expression of TNF-α, MCP-1, IL-6, ROCK1 and ROCK2. Meanwhile, treatment with ripasudil also decreased the apoptosis rates and increased cell viability of these cells. These results indicated that the ripasudil protected the RPE cells from the LPS induced inflammation. The results also suggested that the ripasudil maybe protected the RPE cells from inflammation by targeting the ROCK1 and ROCK2. Therefore, we designed the experiment to further detect the mechanism of ripasudil therapy.

Moreover, there is research revealed that miR-136-5p suppressed the inflammatory response which was induced by the cerebral ischemia/reperfusion [11]. The prediction results of targetscan showed that miR-136-5p may target and regulate the expression of ROCK1 and ROCK2. In this study, we found that the miR-136-5p targeted the promoter region of ROCK1 and ROCK2. The expression of ROCK1 and ROCK2 was promoted after the inhibition of miR-136-5p. Furthermore, the levels of miR-136-5p were

downregulated after the stimulation of LPS and the expression of miR-136-5p was rescued after the treatment of ripasudil. These results indicated that the ripasudil relieved the inflammation damage by upregulating of the miR-136-5p and the higher levels of miR-136-5p targeted and suppressed the levels of ROCK1 and ROCK2.

The NLRP3/ASC/pro-caspase1 was a protein complex which was formed during the process of inflammatory response [13]. The formation of this complex will lead to the production of active caspase-1. After that the active caspase-1 could lead to the upregulation of IL-1β and IL-18. Finally, the higher levels of IL-1β and IL-18 induced the inflammatory response and apoptosis of cells [13, 21, 22]. Furthermore, there is research revealed that the inhibition of ROCK1 and ROCK2 greatly restricted the expression of NLRP3 and therefore suppressed the inflammation which was induced by the higher levels of NLRP3 [23]. In our study, we revealed that the treatment of ripasudil relieved the upregulation of NLRP3, ASC, caspase1, IL-1β and IL-18. After the suppression of miR-136-5p, the levels of these proteins were rescued. Therefore, all the results in this paper implied that the treatment of ripasudil alleviated the inflammation damage of RPE cells by enhanced the level of miR-136-5p and therefore inhibited the expression of ROCK1, ROCK2, NLRP3, ASC, caspase1, IL-1β and IL-18. Finally, the releasing of inflammatory factors declined and the inflammatory injury was relieved. Our study also provided the new approach and target for the treatment of eye diseases which was caused by the inflammation of RPE cells.

Declarations

Ethics approval and consent to participate

Not applicable

Conflict of interest

The authors declare no conflict of interest.

Consent for publication

All authors agreed to publish this paper.

Funding

Not applicable

Authors' contributions

All the authors listed in this paper contributed to the design and completion of the experiments.

Availability of data and materials

The analytical data in this study could be obtained from the corresponding author upon reasonable request.

References

[1] M. Yam, A.L. Engel, Y. Wang, S. Zhu, A. Hauer, R. Zhang, D. Lohner, J. Huang, M. Dinterman, C. Zhao, J.R. Chao, J. Du, Proline mediates metabolic communication between retinal pigment epithelial cells and the retina, J Biol Chem, 294 (2019) 10278-10289.

[2] M.J. Radeke, C.M. Radeke, Y.H. Shih, J. Hu, D. Bok, L.V. Johnson, P.J. Coffey, Restoration of mesenchymal retinal pigmented epithelial cells by TGFbeta pathway inhibitors: implications for age-related macular degeneration, Genome medicine, 7 (2015) 58.

[3] S. Tawara, H. Shimokawa, Progress of the study of rho-kinase and future perspective of the inhibitor, Yakugaku zasshi : Journal of the Pharmaceutical Society of Japan, 127 (2007) 501-514.

[4] H. Shimokawa, A. Takeshita, Rho-kinase is an important therapeutic target in cardiovascular medicine, Arteriosclerosis, thrombosis, and vascular biology, 25 (2005) 1767-1775.

[5] L. Van Aelst, C. D'Souza-Schorey, Rho GTPases and signaling networks, Genes & development, 11 (1997) 2295-2322.

[6] K.P. Garnock-Jones, Ripasudil: first global approval, Drugs, 74 (2014) 2211-2215.

[7] S. Zandi, S. Nakao, K.H. Chun, P. Fiorina, D. Sun, R. Arita, M. Zhao, E. Kim, O. Schueller, S. Campbell, M. Taher, M.I. Melhorn, A. Schering, F. Gatti, S. Tezza, F. Xie, A. Vergani, S. Yoshida, K. Ishikawa, M. Yamaguchi, F. Sasaki, R. Schmidt-Ullrich, Y. Hata, H. Enaida, M. Yuzawa, T. Yokomizo, Y.B. Kim, P. Sweetnam, T. Ishibashi, A. Hafezi-Moghadam, ROCK-isoform-specific polarization of macrophages associated with age-related macular degeneration, Cell reports, 10 (2015) 1173-1186.

[8] R. Yamagishi-Kimura, M. Honjo, T. Komizo, T. Ono, A. Yagi, J. Lee, K. Miyata, T. Fujimoto, T. Inoue, H. Tanihara, J. Nishida, T. Uchida, Y. Araki, M. Aihara, Interaction Between Pilocarpine and Ripasudil on Intraocular Pressure, Pupil Diameter, and the Aqueous-Outflow Pathway, Investigative ophthalmology & visual science, 59 (2018) 1844-1854.

[9] N. Okumura, Y. Okazaki, R. Inoue, K. Kakutani, S. Nakano, S. Kinoshita, N. Koizumi, Effect of the Rho-Associated Kinase Inhibitor Eye Drop (Ripasudil) on Corneal Endothelial Wound Healing, Investigative ophthalmology & visual science, 57 (2016) 1284-1292.

[10] J. Yang, F. Ruan, Z. Zheng, Ripasudil Attenuates Lipopolysaccharide (LPS)-Mediated Apoptosis and Inflammation in Pulmonary Microvascular Endothelial Cells via ROCK2/eNOS Signaling, Medical science monitor : international medical journal of experimental and clinical research, 24 (2018) 3212-3219.

[11] Y. Zhong, C. Yu, W. Qin, LncRNA SNHG14 promotes inflammatory response induced by cerebral ischemia/reperfusion injury through regulating miR-136-5p /ROCK1, Cancer gene therapy, 26 (2019) 234-247.

[12] G. Deng, Y. Gao, Z. Cen, J. He, B. Cao, G. Zeng, S. Zong, miR-136-5p Regulates the Inflammatory Response by Targeting the IKKbeta/NF-kappaB/A20 Pathway After Spinal Cord Injury, Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology, 50 (2018) 512-524.

[13] V. Compan, F. Martin-Sanchez, A. Baroja-Mazo, G. Lopez-Castejon, A.I. Gomez, A. Verkhratsky, D. Brough, P. Pelegrin, Apoptosis-associated speck-like protein containing a CARD forms specks but does not activate caspase-1 in the absence of NLRP3 during macrophage swelling, Journal of immunology (Baltimore, Md. : 1950), 194 (2015) 1261-1273.

[14] K. Mondal, N. Mandal, Role of Bioactive Sphingolipids in Inflammation and Eye Diseases, Advances in experimental medicine and biology, 1161 (2019) 149-167.

[15] W. Zhou, J.H. Quan, F.F. Gao, H. Ismail, Y.H. Lee, G.H. Cha, Modulated Gene Expression of Toxoplasma gondii Infected Retinal Pigment Epithelial Cell Line (ARPE-19) via PI3K/Akt or mTOR Signal Pathway, The Korean journal of parasitology, 56 (2018) 135-145.

[16] K.L. Pennington, M.M. DeAngelis, Epidemiology of age-related macular degeneration (AMD): associations with cardiovascular disease phenotypes and lipid factors, Eye and vision (London, England), 3 (2016) 34.

[17] A. Panday, M.E. Inda, P. Bagam, M.K. Sahoo, D. Osorio, S. Batra, Transcription Factor NF-kappaB: An Update on Intervention Strategies, Archivum immunologiae et therapiae experimentalis, 64 (2016) 463-483.

[18] K. Schweitzer, P.M. Bozko, W. Dubiel, M. Naumann, CSN controls NF-kappaB by deubiquitinylation of IkappaBalpha, The EMBO journal, 26 (2007) 1532-1541.

[19] T. Komizo, T. Ono, A. Yagi, K. Miyata, M. Aihara, Additive intraocular pressure-lowering effects of the Rho kinase inhibitor ripasudil in Japanese patients with various subtypes of glaucoma, Japanese journal of ophthalmology, 63 (2019) 40-45.

[20] M. Yasuda, K. Takayama, T. Kanda, M. Taguchi, H. Someya, M. Takeuchi, Comparison of intraocular pressure-lowering effects of ripasudil hydrochloride hydrate for inflammatory and corticosteroid-induced ocular hypertension, PLoS One, 12 (2017) e0185305.

[21] D. Li, W. Ren, Z. Jiang, L. Zhu, Regulation of the NLRP3 inflammasome and macrophage pyroptosis by the p38 MAPK signaling pathway in a mouse model of acute lung injury, Molecular medicine reports, 18 (2018) 4399-4409.

[22] Q. Wang, R. Imamura, K. Motani, H. Kushiyama, S. Nagata, T. Suda, Pyroptotic cells externalize eat-me and release find-me signals and are efficiently engulfed by macrophages, International immunology, 25 (2013) 363-372.

[23] S. Kanno, S. Hirano, S. Chiba, H. Takeshita, T. Nagai, M. Takada, K. Sakamoto, T. Mukai, The role of Rho-kinases in IL-1beta release through phagocytosis of fibrous particles in human monocytes, Archives of toxicology, 89 (2015) 73-85.

Figures

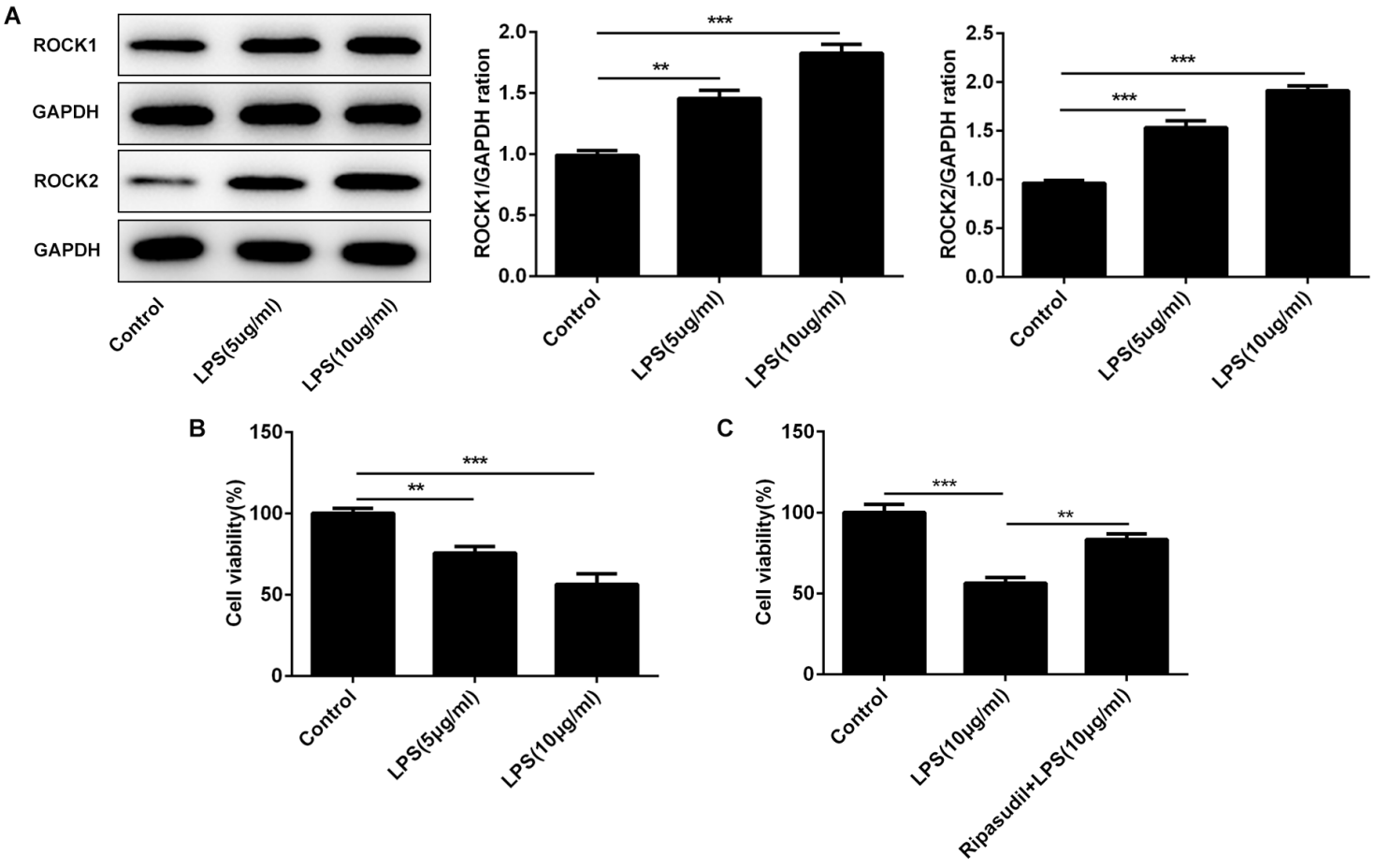

Figure 1

Ripasudil relieved the LPS induced inflammatory response in RPE cells. (A) Western-blotting were used to determine the expression of ROCK1 and ROCK2 after the stimulation of LPS. (B) The cell viability of RPE cells was detected with the CCK-8 after the stimulation of LPS. (C) The cell viability of RPE cells was determined after the treatment of ripasudil. *p<0.05, **p<0.01, ***p<0.001.

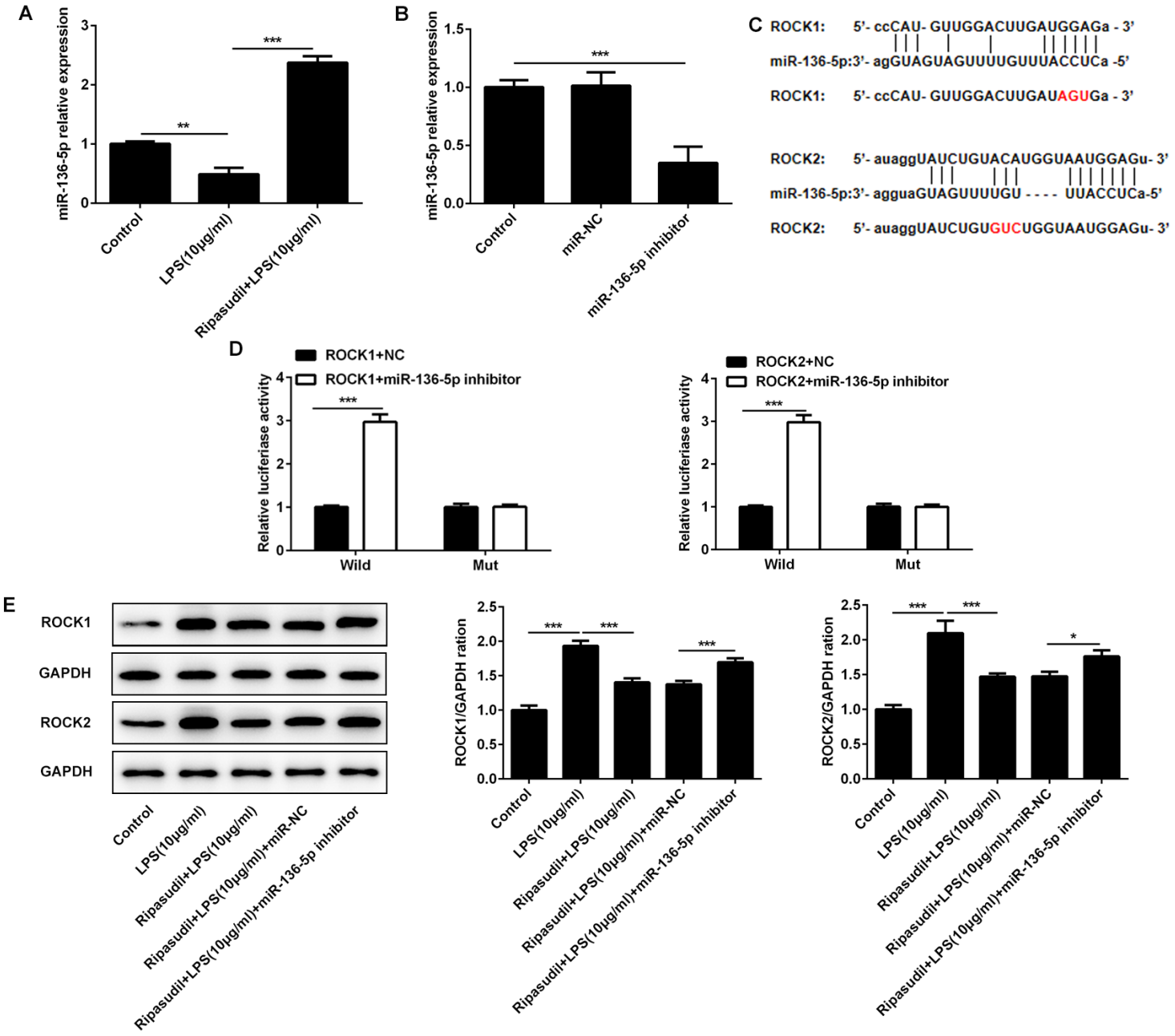

Figure 2

Ripasudil alleviated the inflammatory damage by mediating the expression of miR-136-5p. (A) The relative expression of miR-136-5p in RPE cells was detected with the RT-PCR. (B) The levels of miR-136-5p in RPE cells was determined after the application of miR-136-5p inhibitor. (C) The sequences of mature miR-136-5p and putative target sites of wild type ROCK1 and ROCK2. The mutant sequences of ROCK1 and ROCK2 was marked with red color. (D) Luciferase activity was determined in the miR-136-5p inhibitor and negative control groups. (E) The protein levels of ROCK1 and ROCK2 were determined after the application of miR-136-5p. *p<0.05, **p<0.01, ***p<0.001.

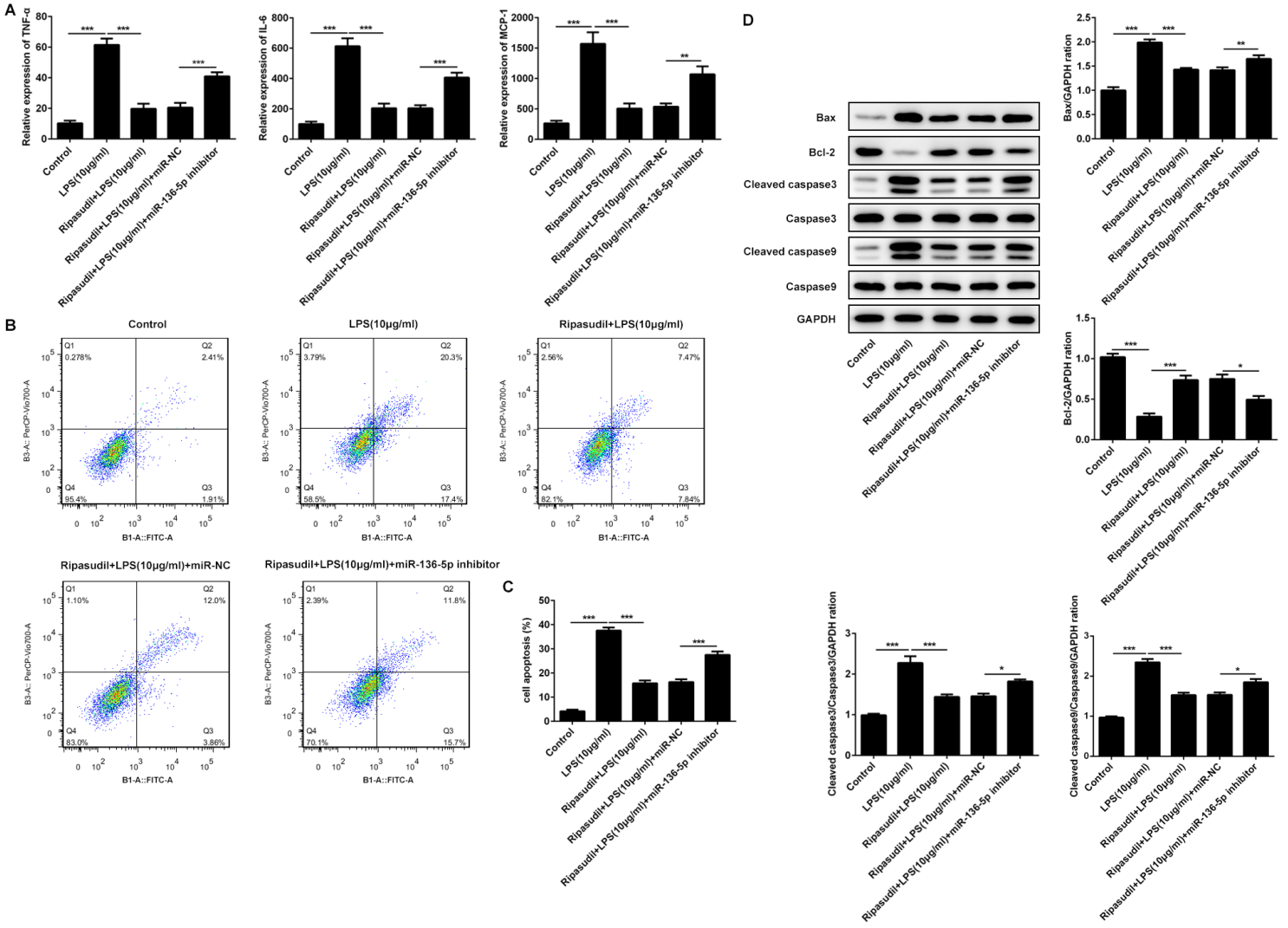

Figure 3

Suppression of miR-136-5p lead to the upregulation of inflammatory factors and increasing apoptosis ratios. (A) The levels of TNF-α, IL-6 and MCP-1 in the supernatant were measured with the ELISA assays. (B) The apoptosis rates of RPE cells was determined with the flow cytometry. (C) The quantitative data of apoptosis rates. (D) The expression of apoptosis related proteins (Bax, Bcl-2, Caspase3, Cleaved caspase3, Caspase9 and Cleaved caspase9) in RPE cells was detected with western-blotting. *p<0.05, **p<0.01, ***p<0.001.

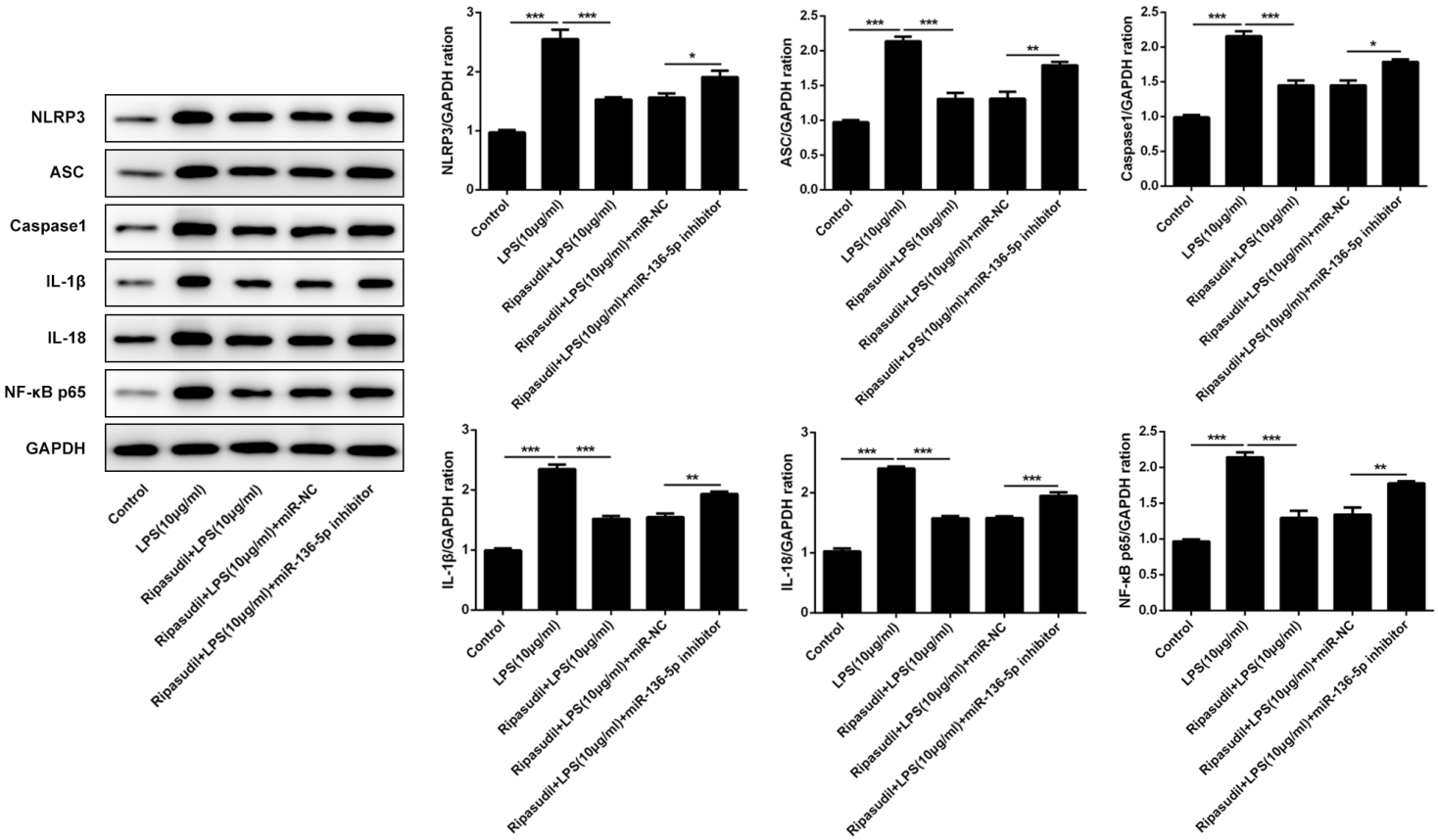

Figure 4

Ripasudil relieved the inflammation induced damage of RPE cells by suppressing the NLRP3/ASC/caspase-1 pathway. The levels of NLRP3, ASC, Caspase1, IL-1β, IL-18 and NF-κB in RPE cells were determined with the western-blotting. The quantitative of these proteins was performed with ImageJ. *p<0.05, **p<0.01, ***p<0.001.