Received: 8 August 2019 | Accepted: 13 January 2020

DOI: 10.1002/jcp.29594

ORIGINAL RESEARCH ARTICLE

Journal of Cellular Physiology WILEY

Differential phosphorylation regulates the shear stress-induced polar activity of Rho-specific guanine nucleotide dissociation inhibitor α

Fei Xie[1] | Shuai Shao[1] | Baohong Zhang[1] | Sha Deng[1] | Aziz Ur Rehman Aziz[1] | Xiaoling Liao[2] | Bo Liu[1]

[1]Liaoning Key Lab of IC & BME System, Dalian University of Technology, Dalian, Liaoning, China

[2]Institute of Biomedical Engineering, Chongqing University of Science and Technology, Chongqing, China

Correspondence
Bo Liu, Liaoning Key Lab of IC & BME System, Dalian University of Technology, Dalian 116024, Liaoning, China.
Email: lbo@dlut.edu.cn

Funding information
National Natural Science Foundation of China, Grant/Award Numbers: 31670867, 31670961

Abstract

The activity of Rho-specific guanine nucleotide dissociation inhibitor α (RhoGDIα) is regulated by its own phosphorylation at different amino acid sites. These phosphorylation sites may have a crucial role in local Rho GTPases activation during cell migration. This paper is designed to explore the influence of phosphorylation on shear stress-induced spatial RhoGDIα activation. Based on the fluorescence resonance energy transfer biosensor sl-RhoGDIα, which was constructed to test the RhoGDIα activity in living cells, new RhoGDIα phosphomimetic mutation (sl-S101E/S174E, sl-Y156E, sl-S101E, sl-S174E) and phosphorylation-deficient mutation (sl-S101A/S174A, sl-Y156A, sl-S101A, sl-S174A) biosensors were designed to test their effects on RhoGDIα activation upon shear stress application in human umbilical vein endothelial cells (HUVECs). The results showed lower RhoGDIα activity at the downstream of HUVECs (the region from the edge of the nucleus to the edge of the cell along with the flow). The overall decrease in RhoGDIα activity was inhibited by Y156A-mutant, whereas the polarized RhoGDIα and Rac1 activity were blocked by S101A/S174A mutant. It is concluded that the Tyr156 phosphorylation mainly mediates shear stress-induced overall RhoGDIα activity, while Ser101/Ser174 phosphorylation mediates its polarization. This study demonstrates that differential phosphorylation of RhoGDIα regulates shear stress-induced spatial RhoGDIα activation, which could be a potential target to control cell migration.

KEYWORDS

phosphorylation, polarity, Rho GTPases, RhoGDIα, shear stress

1 | INTRODUCTION

Fluid shear stress (FSS) directs cell polarity and migration through polarized signaling, including Rho GTPases signaling (Collins & Tzima, 2014; da Silva et al., 2019; Mitchell, Jacobs, Li, Chien, & Kintner, 2007). For example, shear stress-induced polarized Rho GTPases activity is essential for the polar reorientation of the microtubule-organizing center and cell alignment in endothelial cells (Collins & Tzima, 2014). Rho-specific guanine nucleotide dissociation inhibitor α (RhoGDIα) is an ubiquitously expressed member of the RhoGDIs family that is known as a main endogenous negative regulator of Rho GTPases, which influences the membrane-cytoplasm cycle of Rho GTPases (Fujiwara et al., 2016; Hodgson et al., 2016; Li & Lee, 2010; Tkachenko et al., 2011). Polar RhoGDIα can also regulate Rho GTPases activation in a spatiotemporal manner, which is vital for cell directional migration (Hodgson et al., 2016; Lee et al., 2015). Many studies reveal that mechanical stress could affect RhoGDIα expression to regulate the Rho GTPases activity (Pan, Wang, Wang, Chen, & Song, 2014; Qi et al., 2008; Qi et al., 2010; Wu, Song, Li, Zhu, & Pan, 2015). For instance, low shear stress activates Rac1 through

repressing RhoGDIα expression (Qi et al., 2008), while cyclic strain increases Rho GTPases activity via downregulating RhoGDIα expression (Pan et al., 2014; Qi et al., 2010). However, up to now, little is known about the function of RhoGDIα in the polarity of Rho GTPases activity in response to shear stress.

Several mechanisms have been reported to mediate the RhoGDIα activity, such as protein interaction, lipids and post-translational modification (Garcia-Mata, Boulter, & Burridge, 2011; Xie et al., 2017). Thereinto, kinase-mediated phosphorylation of RhoGDIα has been recognized as a vital way for regulating the activity of RhoGDIα (Garcia-Mata et al., 2011; Sabbatini & Williams, 2013; Xie et al., 2017). Sequential phosphorylation of RhoGDIα at Tyr156 and Ser101/Ser174 is coincident with the glucose-induced RhoGDIα-Cdc42 and RhoGDIα-Rac1 dissociation in beta cells (Wang & Thurmond, 2010). In addition, phosphorylation of RhoGDIα by Src at Tyr156 decreases the RhoGDIα activity and promotes RhoGDIα accumulation at membrane ruffling (DerMardirossian, Rocklin, Seo, & Bokoch, 2006), and RhoGDIα returns to cytoplasm from membrane when dephosphorylated by β8 integrin-protein tyrosine phosphatase-PEST complex (Lee et al., 2015). These reports suggest that protein kinases-mediated phosphorylation at different sites of RhoGDIα can alter the activity of Rho GTPases by decreasing the affinity of RhoGDIα for Rho GTPases. To date, whether phosphorylation participates in regulating RhoGDIα activity upon shear stress application is not determined.

FSS has been reported to induce polar Rac1 and Cdc42 activation, but not RhoA (Collins & Tzima, 2014), which may be due to a selective affinity of RhoGDIα for a different member of Rho GTPases. With a newly constructed fluorescence resonance energy transfer (FRET) biosensor, our recent study demonstrated that shear stress can directly reduce RhoGDIα activity in a nonuniform manner, which is relatively independent of the Rho GTPases signaling (Shao et al., 2018). This independent regulatory mechanism determines that the spatial activity of RhoGDIα is vital for Rho GTPases activation and cell migration (Shao et al., 2018; Shcherbakova, Cox Cammer, Huisman, Verkhusha, & Hodgson, 2018). The phosphorylation of RhoGDIα has been found to regulate the location of RhoGDIα and Rho GTPases activities (DerMardirossian et al., 2006). However, whether phosphorylation participates in the regulation of shear stress-mediated RhoGDIα activity is unknown. In the current study, the role of specific phosphorylation of residues, Ser101, Ser174, and Thr156, on RhoGDIα in shear stress-induced spatiotemporal RhoGDIα activity is further investigated. The results suggest that shear stress induces downregulation of RhoGDIα activity in an asymmetric manner, and phosphorylation at different sites regulates the spatial activity of RhoGDIα differently upon shear stress application. Besides, this study reveals that polar RhoGDIα activity regulates shear stress-induced localized Rac1 activation, which is necessary for shear stress-induced cytoskeletal reorganization. This research will help to understand the regulatory mechanism of shear stress-induced endothelial cells polarity and migration.

2 | METHOD

2.1 | Cell culture and shear stress

The cell line of human umbilical vein endothelial cells (HUVECs) was obtained from American Type Culture Collection (ATCC; Eahy926; ATCC). The cells were maintained in high glucose of Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 20% (vol/vol) fetal bovine serum (FBS; Gibco), 1% (vol/vol) penicillin/streptomycin (Gibco) at 37°C in a 5% CO_2 humidified atmosphere. HUVECs were exposed to 20 dyn/cm^2 shear stress in a parallel-plate flow chamber as described previously (Shao et al., 2017).

2.2 | Plasmids and transfection

The sl-RhoGDIα biosensor was constructed by connecting RhoGDIα, the switch II domain of Rho GTPases, a linker and a pair of ECFP and Ypet (Shao et al., 2018). To examine the activity of Tyr156 residues-phosphorylated RhoGDIα, Tyr156 in the RhoGDIα domain of sl-RhoGDIα biosensor was mutated to Glu to monitor phosphomimetic RhoGDIα activity (sl-Y156E), or Ala to monitor the phosphorylation-deficient RhoGDIα activity (sl-Y156A), respectively. Similarly, Ser101 residue was mutated from sl-RhoGDIα biosensor to Glu and Ala to form sl-S101E and sl-S101A, respectively. Similarly, Ser174 residue was mutated to sl-S174E, sl-S174A. Mutant biosensors, sl-S101E/S174E, and sl-S101A/S174A were also prepared by replacing both sites with Glu and Ala, respectively. p21 binding domain (PBD)-GFP consisting of PBD of PAK and GFP was used to detect the distribution of active Rac1 in HUVECs (Shao et al., 2017). DNA plasmids were transfected into cells using Lipofectamine LTX (Invitrogen).

2.3 | Microscope, image acquisition, and analysis

Before imaging experiments, HUVECs expressing biosensors or various exogenous proteins were cultured on glass coverslips and starved with DMEM supplemented with 0.5% FBS for at least 12 hr. Isolated single-cell images were collected by an inverted microscope (Nikon Eclipse Ti Series, Ti-FI Epi-fl/1, Nikon, Tokyo, Japan) and a cold CCD (Evolve-512-M-FW-16-AC, Photometrics, Tucson, AZ) using MetaMorph software (Universal Imaging, Downingtown, PA) with a 440DF20 excitation filter, a 455DRLP dichroic mirror and two emission filters controlled by a filter controller (480DF30 for ECFP and 535DF25 for YPet). The pixel-by-pixel ratio images were produced after background subtraction using MetaFluor software (Universal Imaging). The FRET ratio, which is calculated by FRET/ECFP emission value, were quantified and analyzed by Excel (Microsoft, Redmond, WA).

The polarity of FRET ratio images and fluorescence images were analyzed as described previously (B. Liu et al., 2014). In brief, the FRET ratio images of single-cell were divided into three regions, including upstream (region from the edge of cell to the edge of nucleus along the

flow), midstream (region between the two edges of nucleus along the flow) and downstream (region from the edge of nucleus to the edge of cell along the flow) according to the FSS direction. The FRET ratio of the three regions and the whole cell were quantified by using MetaFluor (Universal Imaging). To analyze the polar distribution of active Rac1, the fluorescence images were processed by Matlab (Mathworks). The single-cell was segmented from the images by using Otsu's method and equally divided into 50 regions along the shear stress direction after background subtraction (Shao et al., 2017). The percentage of fluorescence intensity in each region occupying the overall was normalized to the same level before shear stress application. The normalized percentage of fluorescence intensity data from different cells in the same group was averaged and drawn into three-dimensional images along the time axis. The first 15 regions and last 15 regions were selected as downstream and upstream, respectively.

2.4 | Statistical analysis

All data were normalized by the value before shear stress stimulation in the same cell. Student's *t* test was used to compare the polar difference between the upstream region and downstream region in the same group, and the overall FRET ratio difference between two groups. The *p* value (<.05) showed a significant difference (two-tailed *t* test).

3 | RESULTS

3.1 | Shear stress inhibits the activity of RhoGDIα in a polarized manner

Since Rho GTPases activation is dependent on the disassociation of Rho GTPases from the RhoGDIα-Rho GTPase complex (Shcherbakova et al., 2018), the regulation of RhoGDIα activity by shear stress is investigated in HUVECs. The image of single-cell was divided into upstream, midstream, downstream as a previous report described (B. Liu et al., 2014). The region between the edge of nucleus facing the flow and the edge of cell facing the flow is selected as upstream, the region around the nucleus is selected as midstream, and the region between the edge of nucleus along the flow and the edge of cell along the flow is selected as downstream (Figure 1a). A RhoGDIα FRET biosensor (sl-RhoGDIα) was employed to visualize the RhoGDIα activity by detecting the binding degree of RhoGDIα to the Rho GTPases Switch II domain (Shao et al., 2018). The FRET ratio, which represents the RhoGDIα activity, decreases obviously upon 20 dyn/cm^2 shear stress application within 30 min (Figure 1a,b). In addition, it decreases more in the downstream region than the upstream region within the cell ($p < .05$; Figure 1c), indicating that shear stress induces a stronger inhibition of RhoGDIα activity in the downstream region of HUVECs.

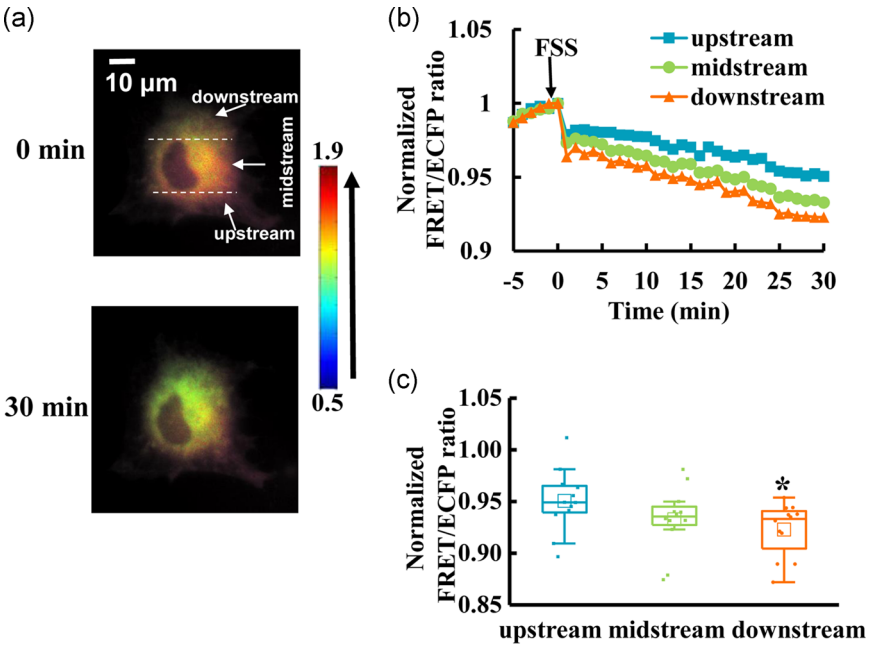

FIGURE 1 Shear stress inhibits RhoGDIα activity in a polar manner. (a) The representative FRET ratio images of the sl-RhoGDIα biosensor under 20 dyn/cm^2 of shear stress. The arrow shows the direction of shear stress. (b) The averaged time courses of the FRET/ECFP emission ratios of a sl-RhoGDIα biosensor at upstream, midstream and downstream of HUVECs upon 20 dyn/cm^2 shear stress application within 30 min ($n = 12$). (c) Averaged FRET/ECFP emission ratios of a sl-RhoGDIα biosensor at upstream, midstream and downstream of HUVECs upon 20 dyn/cm^2 shear stress application after 30 min (*$p < .05$ when compared to the upstream). RhoGDIα, Rho-specific guanine nucleotide dissociation inhibitor α

3.2 | The polarity of RhoGDIα activity in response to shear stress is regulated by PAK1-induced RhoGDIα Ser101/Ser174 phosphorylation

To determine whether PAK1-mediated RhoGDIα phosphorylation participates in the polarized RhoGDIα activity in response to shear stress, HUVECs transfected with sl-RhoGDIα biosensor are pretreated with 30 μM IPA-3 (PAK1 inhibitor; Abcam, UK) for 10 min to inhibit the PAK1 activity before shear stress application. The shear stress-induced decrease of the sl-RhoGDIα FRET ratio is depressed significantly ($p < .05$) by IPA-3 pretreatment (Figure 2b,c). However, no difference is seen between upstream and downstream of IPA-3 treated HUVECs ($p > .05$; Figure 2d). It indicates that inhibition of PAK1 activity blocks both overall and polarized RhoGDIα activity.

Since PAK1 has been reported to phosphorylate RhoGDIα at Ser101 and Ser174 sites (Chong, Tan, Lim, & Manser, 2001; DerMardirossian, Schnelzer, & Bokoch, 2004), sl-S101A/S174A and sl-S101E/S174E biosensors are employed to further investigate the role of RhoGDIα Ser101/Ser174 phosphorylation in shear stress-caused polar RhoGDIα activity. The decrease of overall FRET ratio in sl-S101A/S174A (RhoGDIα Ser101/Ser174 phosphorylation-deficient mutant) group is found lower than sl-RhoGDIα and IPA-3 group after 30 min of shear stress application ($p < .05$; Figure 2a–c). While no significant difference in its FRET ratio is observed between the upstream and downstream ($p > .05$), which is similar to the IPA-3 group (Figure 2d). In contrast, the FRET ratio of sl-S101E/S174E at the upstream is higher than downstream ($p < .05$), although no obvious difference ($p > .05$) of its overall ratio is found (Figures 2b and 2d). Taken together, these results indicate that shear stress-induced polar RhoGDIα activity is mediated by PAK1-induced RhoGDIα Ser101/Ser174 phosphorylation.

3.3 | Both Ser101 and Ser174 individually participate in the regulation of shear stress-induced polar RhoGDIα activity

Since some kinases such as PKA can only phosphorylate Ser174 in RhoGDIα (Oishi, Makita, Sato, & Iiri, 2012), the role of phosphorylation of RhoGDIα at Ser174 and Ser101 in RhoGDIα activity in response to shear stress is further investigated. sl-S101A, sl-S101E, sl-S174A, and sl-S174E biosensors are transfected to HUVECs to explore the effects of phosphorylation of Ser101 and Ser174, respectively, on shear stress-induced RhoGDIα activity. After 30 min of shear stress application, the FRET ratio in sl-S101A is found higher than in sl-S101E and sl-RhoGDIα ($p < .05$; Figure 3a,b). However, the

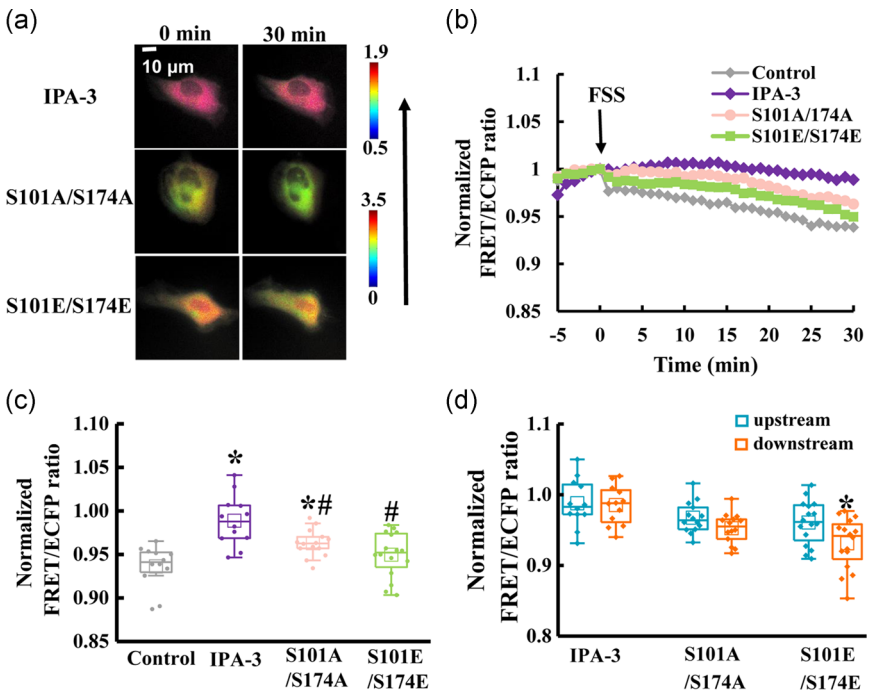

FIGURE 2 PAK1 regulates shear stress-caused polar RhoGDIα activity. The representative FRET ratio images (a) and averaged time courses (b) of the IPA-3 pretreated sl-RhoGDIα ($n = 12$), sl-S101A/S174A ($n = 14$) and sl-S101E/S174E ($n = 16$) biosensors to represent the activity of RhoGDIα and its mutants upon 20 dyn/cm^2 shear stress application within 30 min. (c) Averaged values of overall FRET/ECFP emission ratios of the IPA-3 pretreated sl-RhoGDIα, sl-S101A/S174A, and sl-S101E/S174E biosensors upon 20 dyn/cm^2 shear stress application after 30 min (*$p < .05$ when comparing to the control group, $^{\#}p < .05$ when comparing to the IPA-3 group). (d) Averaged FRET/ECFP emission ratio values at upstream and downstream of the IPA-3 pretreated sl-RhoGDIα, sl-S101A/S174A, and sl-S101E/S174E biosensors upon 20 dyn/cm^2 shear stress application after 30 min (*$p < .05$ when compared to the upstream). FRET, fluorescence resonance energy transfer; RhoGDIα, Rho-specific guanine nucleotide dissociation inhibitor α

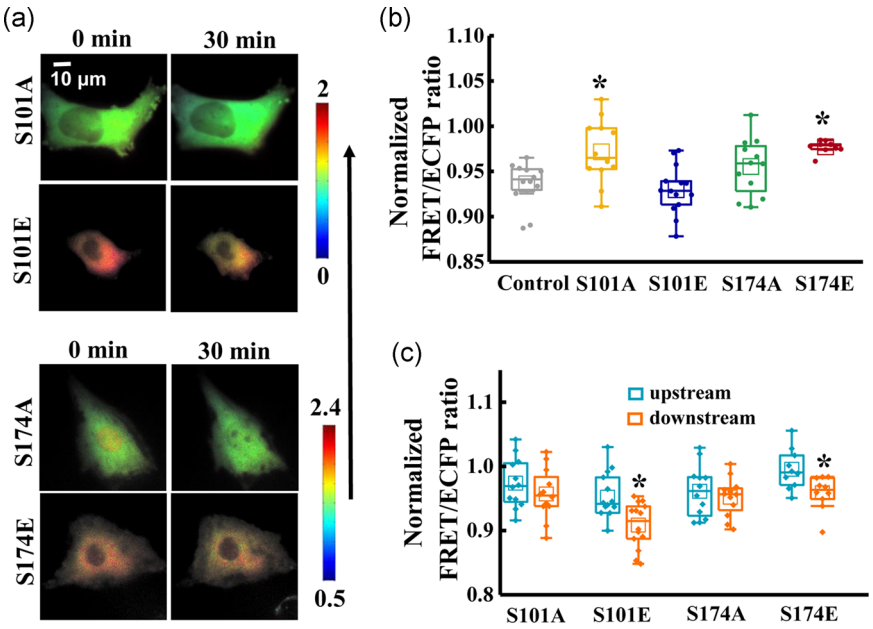

FIGURE 3 Shear stress-caused polar inhibition of RhoGDIα activity is dependent on RhoGDIα Ser101 and Ser174 phosphorylation. The representative FRET ratio images (a) of the sl-S101A (n = 12), sl-S101E (n = 14), sl-S174A (n = 12), and sl-S174E (n = 10) biosensors to represent the activity of RhoGDIα and its mutants upon 20 dyn/cm^2 shear stress application within 30 min. (b) Averaged values of overall FRET/ECFP emission ratios of the sl-S101A, sl-S101E, sl-S174A, and sl-S174E biosensors upon 20 dyn/cm^2 shear stress application after 30 min (*p < .05 when comparing to the control group). (c) Averaged FRET/ECFP emission ratios values at upstream and downstream of the sl-S101A, sl-S101E, sl-S174A, and sl-S174E biosensors upon 20 dyn/cm^2 shear stress application after 30 min (*p < .05 when compared to the upstream). FRET, fluorescence resonance energy transfer; RhoGDIα, Rho-specific guanine nucleotide dissociation inhibitor α

FRET ratio of sl-S174E, but not sl-S174A, is higher than sl-RhoGDIα (p < .05; Figure 3a,b). These results suggest that the interaction of RhoGDIα for Rho GTPases is protected by RhoGDIα Ser174 phosphorylation, whereas inhibited by RhoGDIα Ser101 phosphorylation in response to shear stress. On the other hand, there is no difference in the FRET ratio between upstream and downstream of HUVECs expressing both sl-S101A and sl-S174A biosensors (p > .05; Figure 3c). Furthermore, it is found that the FRET ratio of both sl-S101E and sl-S174E biosensors at the downstream is lower than upstream (p < .05; Figure 3c). These results demonstrate that shear stress-induced polar RhoGDIα activity is regulated by phosphorylation of both Ser101 and Ser174 separately.

3.4 | The phosphorylation of RhoGDIα at Tyr156 regulates the overall RhoGDIα activity

Single Tyr156 phosphorylation of RhoGDIα decreases its affinity for Rho GTPases and localizes it in membrane ruffles (DerMardirossian et al., 2006). To further investigate the role of RhoGDIα Tyr156 residue phosphorylation in RhoGDIα activity in response to shear stress, sl-Y156A, and sl-Y156E biosensors are employed. As shown in Figure 4b,c, the FRET ratio is found higher in sl-Y156A, whereas lower in sl-Y156E than sl-RhoGDIα after 30 min of shear stress (p < .05). Lower FRET ratio at the downstream of cells is detected in both sl-Y156A and sl-Y156E (p < .05) as compared to the control group (Figures 4a and 4d). These results show that shear stress-caused overall decreased RhoGDIα activity, not the polarity, is mediated by RhoGDIα Tyr156 phosphorylation.

3.5 | Shear stress-caused polarized Rac1 activation is regulated by RhoGDIα Ser101/Ser174 phosphorylation

Since spatiotemporal affinity of RhoGDIα and Rho GTPases is vital for Rho GTPases localized activation during cell migration (Hodgson et al., 2016). Whether RhoGDIα phosphorylation contributes to shear stress-induced Rac1 polarized activation is investigated in this study. PBD-GFP biosensor is employed to detect the distribution of active Rac1. Quantitative analysis shows that the PBD-GFP percentage of fluorescence intensity at the downstream is higher than upstream (p < .05; Figure 5c), suggesting that active Rac1 accumulates at the downstream region of HUVECs after 30 min of 20 dyn/cm^2 shear stress stimulation. RhoGDIα phosphomimetic mutant (S101E/S174E) or phosphorylation-deficient mutant (S101A/S174A) is overexpressed in HUVECs to alter the level of RhoGDIα phosphorylation. The accumulation of active Rac1 at the downstream region along the flow is inhibited by overexpression of S101A/S174A, but not by the S101E/S174E (Figure 5a,b). Similarly, HUVECs pretreated with 30 μM IPA-3 for 10 min show no significant difference (p > .05) between upstream and downstream upon shear stress stimulation

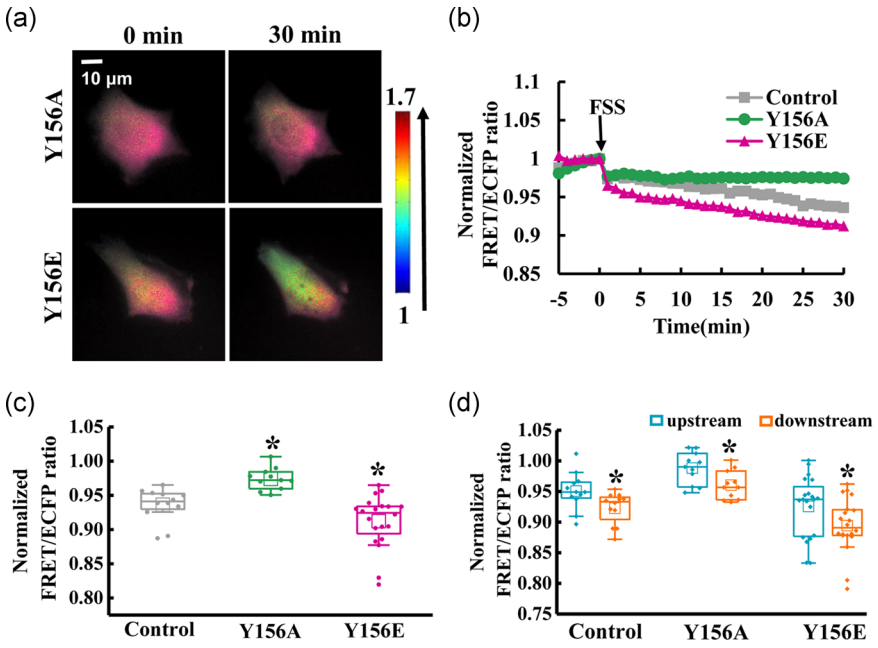

FIGURE 4 Shear stress-caused overall inhibition of RhoGDIα activity is dependent on RhoGDIα Tyr156 phosphorylation. The representative FRET ratio images (a) and averaged time courses (b) of the sl-Y156A ($n = 11$), sl-Y156E ($n = 20$) biosensors to represent the activity of RhoGDIα and its mutants upon 20 dyn/cm^2 shear stress application within 30 min. (c) Averaged values of overall FRET/ECFP emission ratios of the sl-Y156A, sl-Y156E biosensors upon 20 dyn/cm^2 shear stress application after 30 min (*$p < .05$ when comparing to the control group). (d) Averaged FRET/ECFP emission ratio values at upstream and downstream of the sl-Y156A, sl-Y156E biosensors upon 20 dyn/cm^2 shear stress application after 30 min (*$p < .05$ when compared to the upstream). FRET, fluorescence resonance energy transfer; RhoGDIα, Rho-specific guanine nucleotide dissociation inhibitor α

within 30 min (Figure 5c). These results indicate that the local accumulation of active Rac1 requires PAK1-regulated phosphorylation of RhoGDIα at Ser101/Ser174 sites.

3.6 | Shear stress causes polarized Rac1 activation is independent of Tyr156 phosphorylation

Since Rho GTPases activity is regulated by the phosphorylation of RhoGDIα at Ser101, Ser174, and Tyr156 sites (DerMardirossian et al., 2004; DerMardirossian et al., 2006), the role of phosphorylation of RhoGDIα at single Tyr156, Ser101, and Ser174 site in shear stress-induced polarized Rac1 activation is further investigated. Different RhoGDIα mutants (S101A, S174A, Y156A, S101E, S174E, Y156E) are constructed and cotransfected with PBD-GFP to HUVECs to alter phosphorylation level of RhoGDIα. Upon shear stress application within 30 min, PBD-GFP fluorescence is diffusive in cells coexpressing S101A and S174A but accumulated at the downstream of cells coexpressing S101E, S174E, Y156A, or Y156E (Figure 6a,b). The percentage of the fluorescence intensity at the downstream is found higher than upstream in cells coexpressing S101E, S174E, Y156A, or Y156E ($p < .05$), whereas no difference is found in cells coexpressing S101A and S174A ($p > .05$; Figure 6c). These results show that polarized Rac1 activity is inhibited by overexpressing RhoGDIα mutants S174A and S101A, but not by S101E, S174E, Y156A, or Y156E, indicating that shear stress-induced polarized Rac1 activation is regulated by the phosphorylation of RhoGDIα at Ser174 and Ser101, but not at Tyr156.

4 | DISCUSSION

RhoGDIα is the most widely expressed member of RhoGDIs family (Dai et al., 2019), and it regulates all three canonical Rho GTPases through different mechanisms (Hodgson et al., 2016; Xie et al., 2017). Some studies suggested that the phosphorylation of RhoGDIα is a vital mechanism to regulate the spatial interaction of RhoGDIα with Rho GTPases (Hodgson et al., 2016; Shcherbakova et al., 2018). Rho GTPases signaling is influenced by the extracellular environment, and shear stress can mediate RhoGDIα expression and Rac1 activity (Patel et al., 2017; Qi et al., 2008). The role of specific phoshphorylations of RhoGDIα in the regulation of its activity under shear stress is investigated in the present study. The results show that the shear stress-induced overall RhoGDIα activity is mediated by Tyr156 phosphorylation of RhoGDIα, whereas the polarity of RhoGDIα activity is mainly mediated by Ser101 and Ser174 phosphorylation of RhoGDIα.

It is observed that the decreased RhoGDIα activity upon shear stress application is polarized at the downstream region of the HUVECs. This might promote Rho GTPases membrane targeting and RhoGEF-mediated activation since the downregulated affinity of RhoGDIα for Rho GTPases promotes epidermal growth factor-induced Rho GTPases activation (Xiao et al., 2014). It is well-recognized that shear stress regulates Rac1 and Cdc42 activity in a polarized manner, and then promotes cell directional migration (Collins & Tzima, 2014). Lower RhoGDIα activity at the downstream edge of flow in HUVECs is consistent with previous studies that shear stress promotes Rac1 activation at the downstream, whereas depresses the Rac1 activity at

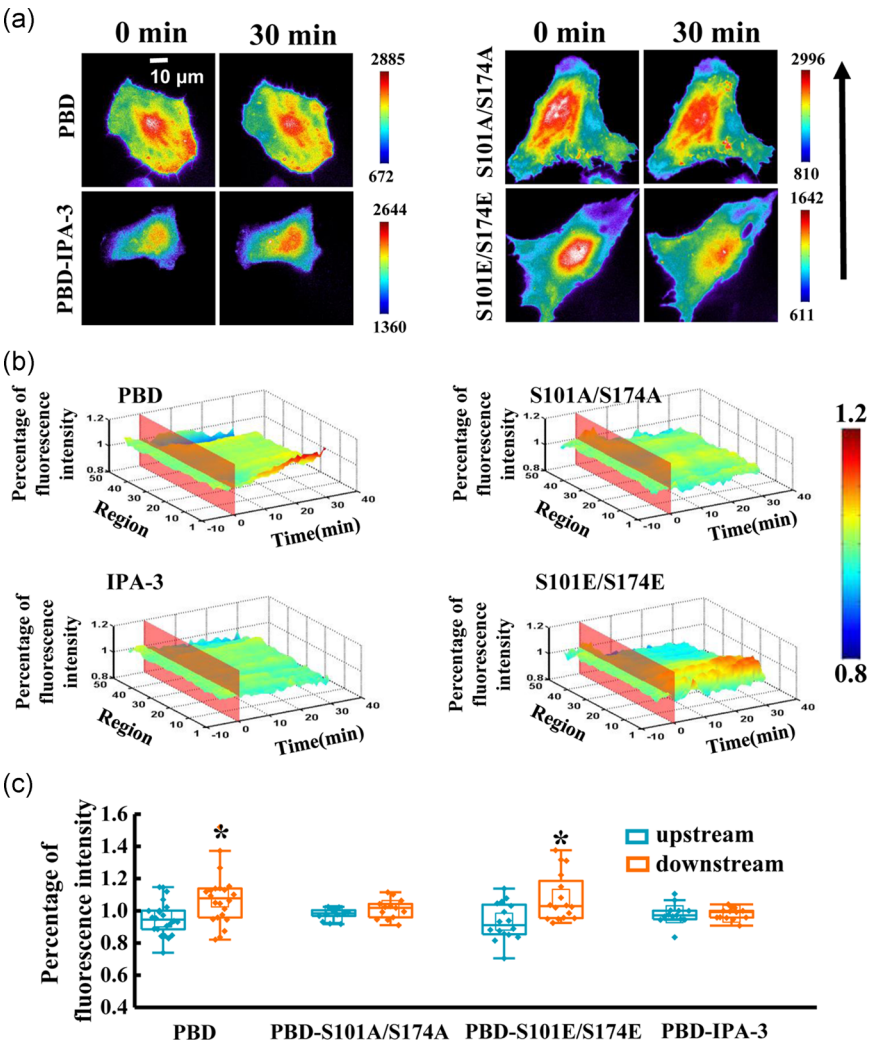

FIGURE 5 Phosphorylation of RhoGDIα by PAK1 regulates the local accumulation of active Rac1. (a) The representative fluorescence images of the PBD-GFP ($n = 22$), IPA-3 pretreated PBD-GFP ($n = 13$), PBD-GFP cotransfected with S101E/S174E ($n = 16$), and PBD-GFP cotransfected with S101A/S174A ($n = 13$) upon 20 dyn/cm^2 shear stress application within 30 min. The arrow shows the direction of shear stress. (b) The average three-dimensional figures in different RhoGDIα phosphorylation states. (c) The normalized fluorescence intensity at upstream and downstream of HUVEC in different RhoGDIα phosphorylation states upon 20 dyn/cm^2 shear stress application after 30 min (*$p < .05$ when compared to the upstream). HUVEC, human umbilical vein endothelial cell; PBD, p21 binding domain; RhoGDIα, Rho-specific guanine nucleotide dissociation inhibitor α

the upstream (Zaidel-Bar, Kam, & Geiger, 2005). In addition, a piece of evidence shows that RhoGDIα has a lower affinity for active Cdc42 than inactive Cdc42 (Johnson, Erickson, & Cerione, 2009). Consequently, it results in lower mobility of active Cdc42 and promotes the active Cdc42 polarization in yeast cells (Woods et al., 2016). It has been reported that integrins disassociate RhoGDIα-Rac complex and promote the interaction of Rho GTPases and effectors, which spatially promotes the clustering of actin at the leading edge of migrating cells (Abramovici et al., 2009; Sun & Barbieri, 2004). Moreover, protrusion/retraction cycles, which are dependent on actin polymerization and myosin contraction (Gagliardi et al., 2015), are influenced by localized dynamics of the RhoGDIα-Rho GTPases complex at the cell edge (Hodgson et al., 2016). Therefore, polar RhoGDIα activity can regulate the reorganization of actin cytoskeletal through controlling the activity of Rho GTPases. Our current study confirms that polar RhoGDIα activity regulates localized Rac1 activation, which is necessary for shear stress-induced cytoskeletal reorganization (Pasapera et al., 2015; Tzima, del Pozo, Shattil, Chien, & Schwartz, 2001). Therefore, polarized RhoGDIα activity may provide spatial cues for Rho GTPases localized activation and directional cell migration. However, our recent study shows inconsistent findings in different cell species, that RhoGDIα has a higher, rather than lower activity at the downstream in response to shear stress in HeLa cells (Shao et al., 2018). It is hypothesized that this opposite polarization may be the consequence of different subcellular localization of focal adhesion proteins between tumor cells and ECs in response to shear stress (Li et al., 2002; Mott & Helmke, 2007; Polacheck, Charest, & Kamm, 2011; Polacheck, German, Mammoto, Ingber, & Kamm, 2014). Paxillin is a focal adhesion protein and can recruit active PAK1 and RhoGEFs through its LD motifs (Rajah et al., 2019; Turner et al., 1999). Previous studies found that shear stress induces upstream polarization of paxillin in tumor cells (Polacheck et al., 2011), while it promotes polarized paxillin activity at the downstream region of ECs (Mott & Helmke, 2007). This difference might induce opposite the subcellular location of PAK1 and then lead to different phosphorylation status of RhoGDIα at the downstream region between tumor cells and ECs.

PAK1 has been reported to be activated by shear stress (Hahn, Orr, Sanders, Jhaveri, & Schwartz, 2009; Jhaveri, Debnath, Chernoff, Sanders, & Schwartz, 2012), and then phosphorylates RhoGDIα at Ser101 and Ser174 sites to promote the disassociation of RhoGDIα and Rac1 (Castro-Castro, Muriel, Del Pozo, & Bustelo, 2016). In addition, Rac1-mediated cytoskeletal reorganization in response to

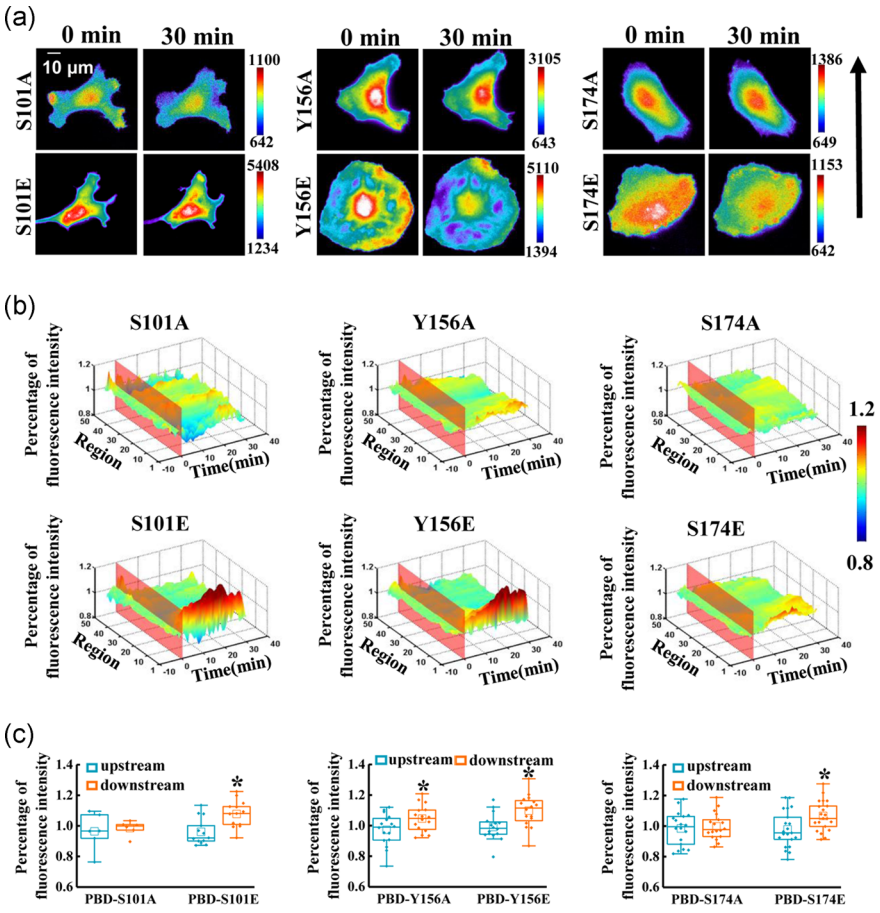

FIGURE 6 Phosphorylation of RhoGDIα at single Ser174 or Ser101 regulates the local accumulation of active Rac1. (a) The representative fluorescence images of the PBD-GFP cotransfected with S101E (n = 13), S101A (n = 6), Y156E (n = 16), Y156A (n = 16), S174E (n = 21), and S174A (n = 18) upon 20 dyn/cm^2 shear stress application within 30 min. The arrow shows the direction of shear stress. (b) The average three-dimensional figures in different RhoGDIα phosphorylation states. (c) The normalized fluorescence intensity at upstream and downstream of HUVEC in different RhoGDIα phosphorylation states upon 20 dyn/cm^2 shear stress application after 30 min (*p < .05 when compared to the upstream). PBD, p21 binding domain; RhoGDIα, Rho-specific guanine nucleotide dissociation inhibitor α

shear stress was found dependent on PAK1 activity (Tzima, 2006). The current results show that shear stress causes polar RhoGDIα activity through PAK1-induced RhoGDIα Ser101/Ser174 phosphorylation. It is speculated that PAK1 may have a polarized activity to enhance downstream RhoGDIα phosphorylation, and then cause lower RhoGDIα activity at the downstream region, because RhoGDIα Ser101/Ser174 phosphorylation-deficient mutant S101A/S174A has no polarized activity after shear stress stimulation. This notion is also supported by the previous reports that PAK1 activity is polarized and restricted to the lamellipodia at the leading edge of migrating cells, and expression of activated PAK1 mutants promote the formation of polarized lamellipodia and cell migration (Dharmawardhane, Brownson, Lennartz, & Bokoch, 1999; Eppinga, Li, Lin, Mak, & Lin, 2006). Since, ECs migrate alone the flow direction in response to shear stress, PAK1 might translocate to the actin-rich lamellipodia and has higher activity at the downstream region of ECs. Subsequently, this may induce RhoGDIα Ser101/Ser174 phosphorylation at the downstream region of ECs. In addition, inhibition of PAK1 activity and S101A/S174A also inhibits active Rac1 accumulation in the direction of flow, which implies that PAK1-mediated RhoGDIα Ser101 and Ser174 phosphorylation might further promote Rac1 activity through reducing RhoGDIα activity at the downstream of ECs. Similarly, recent findings have revealed that RhoGEFs-dependent Rho GTPases activation is rapidly followed by the dissociation of RhoGDIα-Rho GTPases complex at the leading edge of migrating cells (Shcherbakova et al., 2018), and this process is mediated by RhoGDIα phosphorylation (Hodgson et al., 2016; Shcherbakova et al., 2018). These findings suggest that PAK1 determines the spatial phosphorylation of RhoGDIα, which subsequently regulates the RhoGDIα and Rac1 activity in a polar manner (DerMardirossian et al., 2004).

On the other hand, active Rac1 or Cdc42 can bind to the PBD of PAK1, which leads to its autophosphorylation and activation (Pasapera et al., 2015; Whalley et al., 2015). Therefore, it forms positive feedback that maintains the polar RhoGDIα activity and active Rac1 along the direction of flow. RhoGDIα S101A/S174A mutant, which could not be phosphorylated by PAK1, disrupts this positive feedback and shows no polarity at the downstream of the cell. Similarly, it is found that PAK1 is activated and then it restricts the Rac1 activity at the leading edge of migrating cells (Hall, 2005; Sells, Pfaff, & Chernoff, 2000). Shear stress-induced integrin-ECM binding, which activates Rac1 and PAK1, might initiate this positive feedback (del Pozo, Price, Alderson, Ren, & Schwartz, 2000; Tzima et al., 2001). The previous study has also reported that diacylglycerol kinases (DGKs), which is upregulated by integrin activation, generate phosphatidic acid, and then activates PAK1 and promotes the release of Rac1 from RhoGDIα in response to PDGF stimulation (Abramovici et al., 2009). DGKs can also recruit PKCα to phosphorylate RhoGDIα at Ser34 site, and promote dissociation of RhoA from RhoGDIα (Ard et al., 2012). Hence, integrin might initiate this positive feedback through DGKs and PAK1 pathways. Whether PAK1 and DGKs also have a polarized pattern under shear stress still needs to be explored.

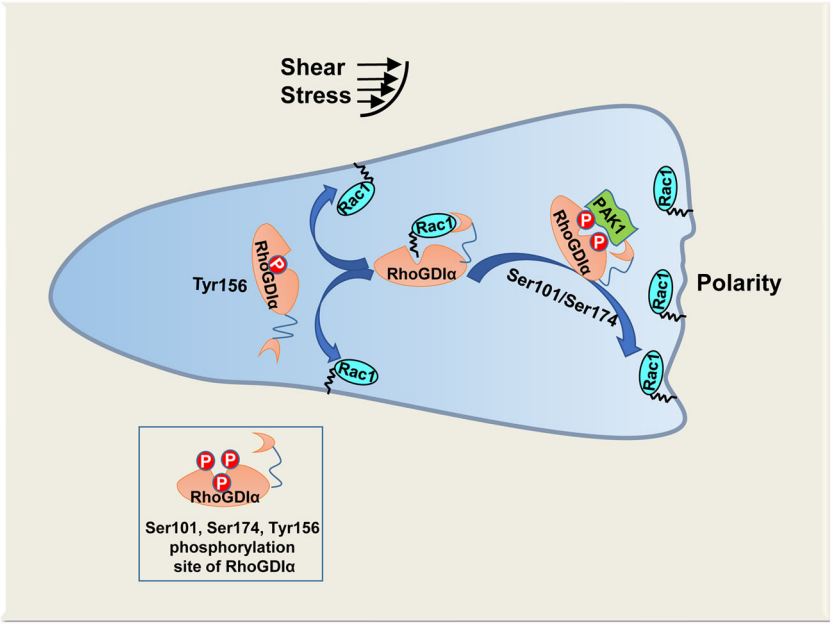

FIGURE 7 Proposed model depicting how differential phosphorylation regulates shear stress-induced RhoGDIα activity. RhoGDIα Y156 phosphorylation mainly mediates overall RhoGDIα activity, and RhoGDIα S101/S174 phosphorylation mediates its polarization and the downstream of Rac1 activation upon shear stress application. RhoGDIα, Rho-specific guanine nucleotide dissociation inhibitor α

Evidence has shown that Ser174 can be phosphorylated by PKA alone and then regulates Rho GTPases activity (Xiao et al., 2014), which implies that Ser101 and Ser174 may have different contribution to the regulation of RhoGDIα activity. In contrast to the phosphorylation of Ser101/Ser174 or Ser101 alone, phosphorylation of Ser174 alone negatively regulates shear stress-caused overall downregulated RhoGDIα activity. This result is consistent with previous studies that phosphorylation of RhoGDIα at Ser174 by PKA could not induce the dissociation of Rho GTPases from RhoGDIα-Rho GTPases, and even negatively regulates RhoA activity (Oishi et al., 2012; Qiao et al., 2008; Qiao, Huang, & Lum, 2003). Ser174 locates in the geranylgernayl binding pocket of RhoGDIα, which is vital for the association of Rho GTPases (Hoffman, Nassar, & Cerione, 2000). It could be the reason that phosphorylation of RhoGDIα at Ser174 alone induces the conformational change in geranylgeranyl binding pocket of RhoGDIα, which protects RhoGDIα-Rho GTPases interaction in response to shear stress. In addition, both Ser101 and Ser174 phosphorylation regulate the shear stress-induced polar RhoGDIα and Rac1 activity. These results are consistent with the observation that both Ser101 and Ser174 residues are important for the dissociation of Rho GTPases (DerMardirossian et al., 2004). It is possible that Ser101 and Ser174 phosphorylation will promote the binding of RhoGDIα dissociation factors at the downstream of cells. This notion is underscored by the observation that 14-3-3τ binding to Ser174-phosphorylated RhoGDIα is to promote Rho GTPases activation, and the status of Ser101 residue also affects the interaction of RhoGDIα and 14-3-3τ (Xiao et al., 2014).

The phosphorylation of RhoGDIα at Tyr156 by Src is a vital way for Rho GTPases disassociation from RhoGDIα and it is also involved in directed cell migration (DerMardirossian et al., 2006; Lee et al., 2015). The dynamics of RhoGDIα-Cdc42 interactions and localized Cdc42 activation at cell protrusion are regulated by the Src-mediated RhoGDIα phosphorylation (Hodgson et al., 2016). The current results show that the phosphorylation of RhoGDIα at Tyr156 promotes the shear stress-induced overall downregulation of RhoGDIα activity, while inhibition of Tyr156 phosphorylation mainly inhibits the shear stress-induced overall, but not polar RhoGDIα activity. These results imply that Tyr156 phosphorylation can function as a switch to control the overall rather than polar RhoGDIα activity in response to shear stress. Consistently, neither Y156A nor Y156E influences the polarity of active Rac1. Previous studies revealed that PDGF causes both Rac and Src activation, and only Rac activity can be polarized at the leading edge of migrating cells (Ouyang, Sun, Chien, & Wang, 2008). Our previous study found that high shear stress (65 dyn/cm^2) induces polarized Src activation at the upstream of cells, while no polarity is found under the physiological level of shear stress (B. Liu et al., 2014). This might lead to uniform phosphorylation of RhoGDIα at Tyr156 by Src in response to the physiological level of shear stress. Consistently, shear stress-induced Src-dependent Vav2 phosphorylation can lead to Rac1 overall, but not polar activation (Collins & Tzima, 2014; Y. Liu et al., 2013; Tzima et al., 2005). Therefore, Src might cause uniform phosphorylation of RhoGDIα at Tyr156, which contributes to overall, but not polar Rho GTPases activation. The mechanism still needs to be elucidated.

5 | CONCLUSION

These results imply that RhoGDIα Tyr156 sites might function as a switch for the regulation of overall RhoGDIα activity, which might further mediate the Rho GTPases membrane-cytoplasm cycle; and the phosphorylation of RhoGDIα at Ser101/Ser174 sites regulates polar RhoGDIα activity, which is associated with localized Rho GTPases activity (Figure 7). Whether the shear stress-induced

polarity of several other molecules or structures, including FAK, actin and focal adhesion (Zhang et al., 2019), is also mediated by the phosphorylation of RhoGDIα will be worth investigating. However, this study illustrates a RhoGDIα phosphorylation-dependent mechanism for regulating Rho GTPases activity, which might be vital in atherogenesis or repair process after vascular injury, and it also provides a deep understanding of cellular mechanotransduction.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China [grant numbers 31670867, 31670961].

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

F. X. and B. L. designed the research of this paper; F. X., S. S., B. H. Z., and S. D. performed research and analyzed data; F. X., A. U. R. A., X. L. L., and B. L. wrote the paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Bo Liu http://orcid.org/0000-0002-5375-0505

REFERENCES

Abramovici, H., Mojtabaie, P., Parks, R. J., Zhong, X. P., Koretzky, G. A., Topham, M. K., & Gee, S. H. (2009). Diacylglycerol kinase zeta regulates actin cytoskeleton reorganization through dissociation of Rac1 from RhoGDI. *Molecular Biology of the Cell*, *20*(7), 2049–2059. https://doi.org/10.1091/mbc.E07-12-1248

Ard, R., Mulatz, K., Abramovici, H., Maillet, J. C., Fottinger, A., Foley, T., … Gee, S. H. (2012). Diacylglycerol kinase zeta regulates RhoA activation via a kinase-independent scaffolding mechanism. *Molecular Biology of the Cell*, *23*(20), 4008–4019. https://doi.org/10.1091/mbc.E12-01-0026

Castro-Castro, A., Muriel, O., Del Pozo, M. A., & Bustelo, X. R. (2016). Characterization of novel molecular mechanisms favoring Rac1 membrane translocation. *PLoS One*, *11*(11):e0166715. https://doi.org/10.1371/journal.pone.0166715

Chong, C., Tan, L., Lim, L., & Manser, E. (2001). The mechanism of PAK activation. Autophosphorylation events in both regulatory and kinase domains control activity. *Journal of Biological Chemistry*, *276*(20), 17347–17353. https://doi.org/10.1074/jbc.M009316200

Collins, C., & Tzima, E. (2014). Rac[e] to the pole: Setting up polarity in endothelial cells. *Small GTPases*, *5*:e28650. https://doi.org/10.4161/sgtp.28650

Dai, F., Qi, Y., Guan, W., Meng, G., Liu, Z., Zhang, T., & Yao, W. (2019). RhoGDI stability is regulated by SUMOylation and ubiquitination via the AT1 receptor and participates in Ang II-induced smooth muscle proliferation and vascular remodeling. *Atherosclerosis*, *288*, 124–136. https://doi.org/10.1016/j.atherosclerosis.2019.07.010

DerMardirossian, C., Rocklin, G., Seo, J. Y., & Bokoch, G. M. (2006). Phosphorylation of RhoGDI by Src regulates Rho GTPase binding and cytosol-membrane cycling. *Molecular Biology of the Cell*, *17*(11), 4760–4768. https://doi.org/10.1091/mbc.E06-06-0533

DerMardirossian, C., Schnelzer, A., & Bokoch, G. M. (2004). Phosphorylation of RhoGDI by Pak1 mediates dissociation of Rac GTPase. *Molecular Cell*, *15*(1), 117–127. https://doi.org/10.1016/j.molcel.2004.05.019

Dharmawardhane, S., Brownson, D., Lennartz, M., & Bokoch, G. M. (1999). Localization of p21-activated kinase 1 (PAK1) to pseudopodia, membrane ruffles, and phagocytic cups in activated human neutrophils. *Journal of Leukocyte Biology*, *66*(3), 521–527.

Eppinga, R. D., Li, Y., Lin, J. L., Mak, A. S., & Lin, J. J. (2006). Requirement of reversible caldesmon phosphorylation at P21-activated kinase-responsive sites for lamellipodia extensions during cell migration. *Cell Motility and the Cytoskeleton*, *63*(9), 543–562. https://doi.org/10.1002/cm.20144

Fujiwara, M., Okamoto, M., Hori, M., Suga, H., Jikihara, H., Sugihara, Y., … Tatsuka, M. (2016). Radiation-induced RhoGDIbeta cleavage leads to perturbation of cell polarity: A possible link to cancer spreading. *Journal of Cellular Physiology*, *231*(11), 2493–2505. https://doi.org/10.1002/jcp.25362

Gagliardi, P. A., Puliafito, A., di Blasio, L., Chianale, F., Somale, D., Seano, G., … Primo, L. (2015). Real-time monitoring of cell protrusion dynamics by impedance responses. *Scientific Reports*, *5*, 10206. https://doi.org/10.1038/srep10206

Garcia-Mata, R., Boulter, E., & Burridge, K. (2011). The "invisible hand": Regulation of RHO GTPases by RHOGDIs. *Nature Reviews Molecular Cell Biology*, *12*(8), 493–504. https://doi.org/10.1038/nrm3153

Hahn, C., Orr, A. W., Sanders, J. M., Jhaveri, K. A., & Schwartz, M. A. (2009). The subendothelial extracellular matrix modulates JNK activation by flow. *Circulation Research*, *104*(8), 995–1003. https://doi.org/10.1161/CIRCRESAHA.108.186486

Hall, A. (2005). Rho GTPases and the control of cell behaviour. *Biochemical Society Transactions*, *33*(Pt 5), 891–895. https://doi.org/10.1042/BST20050891

Hodgson, L., Spiering, D., Sabouri-Ghomi, M., Dagliyan, O., DerMardirossian, C., Danuser, G., & Hahn, K. M. (2016). FRET binding antenna reports spatiotemporal dynamics of GDI-Cdc42 GTPase interactions. *Nature Chemical Biology*, *12*(10), 802–809. https://doi.org/10.1038/nchembio.2145

Hoffman, G. R., Nassar, N., & Cerione, R. A. (2000). Structure of the Rho family GTP-binding protein Cdc42 in complex with the multifunctional regulator RhoGDI. *Cell*, *100*(3), 345–356.

Jhaveri, K. A., Debnath, P., Chernoff, J., Sanders, J., & Schwartz, M. A. (2012). The role of p21-activated kinase in the initiation of atherosclerosis. *BMC Cardiovascular Disorders*, *12*, 55. https://doi.org/10.1186/1471-2261-12-55

Johnson, J. L., Erickson, J. W., & Cerione, R. A. (2009). New insights into how the Rho guanine nucleotide dissociation inhibitor regulates the interaction of Cdc42 with membranes. *Journal of Biological Chemistry*, *284*(35), 23860–23871. https://doi.org/10.1074/jbc.M109.031815

Lee, H. S., Cheerathodi, M., Chaki, S. P., Reyes, S. B., Zheng, Y., Lu, Z., … McCarty, J. H. (2015). Protein tyrosine phosphatase-PEST and beta8 integrin regulate spatiotemporal patterns of RhoGDI1 activation in migrating cells. *Molecular and Cellular Biology*, *35*(8), 1401–1413. https://doi.org/10.1128/MCB.00112-15

Li, S., Butler, P., Wang, Y., Hu, Y., Han, D. C., Usami, S., … Chien, S. (2002). The role of the dynamics of focal adhesion kinase in the mechanotaxis of endothelial cells. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(6), 3546–3551. https://doi.org/10.1073/pnas.052018099

Li, X., & Lee, A. Y. (2010). Semaphorin 5A and plexin-B3 inhibit human glioma cell motility through RhoGDIalpha-mediated inactivation of Rac1 GTPase. *Journal of Biological Chemistry*, *285*(42), 32436–32445. https://doi.org/10.1074/jbc.M110.120451

Liu, Y., Collins, C., Kiosses, W. B., Murray, A. M., Joshi, M., Shepherd, T. R., … Tzima, E. (2013). A novel pathway spatiotemporally activates Rac1 and redox signaling in response to fluid shear stress. *Journal of Cell Biology*, *201*(6), 863–873. https://doi.org/10.1083/jcb.201207115

Liu, B., Lu, S., Hu, Y. L., Liao, X., Ouyang, M., & Wang, Y. (2014). RhoA and membrane fluidity mediates the spatially polarized Src/FAK activation in response to shear stress. *Scientific Reports*, *4*, 7008. https://doi.org/10.1038/srep07008

Mitchell, B., Jacobs, R., Li, J., Chien, S., & Kintner, C. (2007). A positive feedback mechanism governs the polarity and motion of motile cilia. *Nature*, *447*(7140), 97–101. https://doi.org/10.1038/nature05771

Mott, R. E., & Helmke, B. P. (2007). Mapping the dynamics of shear stress-induced structural changes in endothelial cells. *American Journal of Physiology—Cell Physiology*, *293*(5), C1616–C1626. https://doi.org/10.1152/ajpcell.00457.2006

Oishi, A., Makita, N., Sato, J., & Iiri, T. (2012). Regulation of RhoA signaling by the cAMP-dependent phosphorylation of RhoGDIalpha. *Journal of Biological Chemistry*, *287*(46), 38705–38715. https://doi.org/10.1074/jbc.M112.401547

Ouyang, M., Sun, J., Chien, S., & Wang, Y. (2008). Determination of hierarchical relationship of Src and Rac at subcellular locations with FRET biosensors. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(38), 14353–14358. https://doi.org/10.1073/pnas.0807537105

Pan, J., Wang, T., Wang, L., Chen, W., & Song, M. (2014). Cyclic strain-induced cytoskeletal rearrangement of human periodontal ligament cells via the Rho signaling pathway. *PLoS One*, *9*(3):e91580. https://doi.org/10.1371/journal.pone.0091580

Pasapera, A. M., Plotnikov, S. V., Fischer, R. S., Case, L. B., Egelhoff, T. T., & Waterman, C. M. (2015). Rac1-dependent phosphorylation and focal adhesion recruitment of myosin IIA regulates migration and mechanosensing. *Current Biology*, *25*(2), 175–186. https://doi.org/10.1016/j.cub.2014.11.043

Patel, R., Sriramoji, S., Marucci, M., Aziz, I., Shah, S., & Sesti, F. (2017). Cytoskeletal remodeling via Rho GTPases during oxidative and thermal stress in *Caenorhabditis elegans*. *Biochemical and Biophysical Research Communications*, *492*(3), 338–342. https://doi.org/10.1016/j.bbrc.2017.08.112

Polacheck, W. J., Charest, J. L., & Kamm, R. D. (2011). Interstitial flow influences direction of tumor cell migration through competing mechanisms. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(27), 11115–11120. https://doi.org/10.1073/pnas.1103581108

Polacheck, W. J., German, A. E., Mammoto, A., Ingber, D. E., & Kamm, R. D. (2014). Mechanotransduction of fluid stresses governs 3D cell migration. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(7), 2447–2452. https://doi.org/10.1073/pnas.1316848111

del Pozo, M. A., Price, L. S., Alderson, N. B., Ren, X. D., & Schwartz, M. A. (2000). Adhesion to the extracellular matrix regulates the coupling of the small GTPase Rac to its effector PAK. *The EMBO Journal*, *19*(9), 2008–2014. https://doi.org/10.1093/emboj/19.9.2008

Qi, Y. X., Qu, M. J., Long, D. K., Liu, B., Yao, Q. P., Chien, S., & Jiang, Z. L. (2008). Rho-GDP dissociation inhibitor alpha downregulated by low shear stress promotes vascular smooth muscle cell migration and apoptosis: A proteomic analysis. *Cardiovascular Research*, *80*(1), 114–122. https://doi.org/10.1093/cvr/cvn158

Qi, Y. X., Qu, M. J., Yan, Z. Q., Zhao, D., Jiang, X. H., Shen, B. R., & Jiang, Z. L. (2010). Cyclic strain modulates migration and proliferation of vascular smooth muscle cells via Rho-GDIalpha, Rac1, and p38 pathway. *Journal of Cellular Biochemistry*, *109*(5), 906–914. https://doi.org/10.1002/jcb.22465

Qiao, J., Holian, O., Lee, B. S., Huang, F., Zhang, J., & Lum, H. (2008). Phosphorylation of GTP dissociation inhibitor by PKA negatively regulates RhoA. *American Journal of Physiology—Cell Physiology*, *295*(5), C1161–C1168. https://doi.org/10.1152/ajpcell.00139.2008

Qiao, J., Huang, F., & Lum, H. (2003). PKA inhibits RhoA activation: A protection mechanism against endothelial barrier dysfunction. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *284*(6), L972–L980. https://doi.org/10.1152/ajplung.00429.2002

Rajah, A., Boudreau, C. G., Ilie, A., Wee, T-L., Tang, K., Borisov, A. Z., … Brown, C. M. (2019). Paxillin S273 phosphorylation regulates adhesion dynamics and cell migration through a common protein complex with PAK1 and βPIX. *Scientific Reports*, *9*(1), 11430. https://doi.org/10.1038/s41598-019-47722-3

Sabbatini, M. E., & Williams, J. A. (2013). Cholecystokinin-mediated RhoGDI phosphorylation via PKCalpha promotes both RhoA and Rac1 signaling. *PLoS One*, *8*(6):e66029. https://doi.org/10.1371/journal.pone.0066029

Sells, M. A., Pfaff, A., & Chernoff, J. (2000). Temporal and spatial distribution of activated Pak1 in fibroblasts. *Journal of Cell Biology*, *151*(7), 1449–1458.

Shao, S., Liao, X., Xie, F., Deng, S., Liu, X., Ristaniemi, T., & Liu, B. (2018). FRET biosensor allows spatio-temporal observation of shear stress-induced polar RhoGDIalpha activation. *Communications Biology*, *1*, 224. https://doi.org/10.1038/s42003-018-0232-2

Shao, S., Xiang, C., Qin, K., Ur Rehman Aziz, A., Liao, X., & Liu, B. (2017). Visualizing the spatiotemporal map of Rac activation in bovine aortic endothelial cells under laminar and disturbed flows. *PLoS One*, *12*(11):e0189088. https://doi.org/10.1371/journal.pone.0189088

Shcherbakova, D. M., Cox Cammer, N., Huisman, T. M., Verkhusha, V. V., & Hodgson, L. (2018). Direct multiplex imaging and optogenetics of Rho GTPases enabled by near-infrared FRET. *Nature Chemical Biology*, *14*(6), 591–600. https://doi.org/10.1038/s41589-018-0044-1

da Silva, R. A., Fernandes, C., Feltran, G. D. S., Gomes, A. M., de Camargo Andrade, A. F., Andia, D. C., … Zambuzzi, W. F. (2019). Laminar shear stress-provoked cytoskeletal changes are mediated by epigenetic reprogramming of TIMP1 in human primary smooth muscle cells. *Journal of Cellular Physiology*, *234*(5), 6382–6396. https://doi.org/10.1002/jcp.27374

Sun, J., & Barbieri, J. T. (2004). ExoS Rho GTPase-activating protein activity stimulates reorganization of the actin cytoskeleton through Rho GTPase guanine nucleotide disassociation inhibitor. *Journal of Biological Chemistry*, *279*(41), 42936–42944. https://doi.org/10.1074/jbc.M406493200

Tkachenko, E., Sabouri-Ghomi, M., Pertz, O., Kim, C., Gutierrez, E., Machacek, M., … Ginsberg, M. H. (2011). Protein kinase A governs a RhoA-RhoGDI protrusion-retraction pacemaker in migrating cells. *Nature Cell Biology*, *13*(6), 660–667. https://doi.org/10.1038/ncb2231

Turner, C. E., Brown, M. C., Perrotta, J. A., Riedy, M. C., Nikolopoulos, S. N., McDonald, A. R., … Leventhal, P. S. (1999). Paxillin LD4 motif binds PAK and PIX through a novel 95-kD ankyrin repeat, ARF–GAP protein: A role in cytoskeletal remodeling. *The Journal of Cell Biology*, *145*(4), 851–863. https://doi.org/10.1083/jcb.145.4.851

Tzima, E. (2006). Role of small GTPases in endothelial cytoskeletal dynamics and the shear stress response. *Circulation Research*, *98*(2), 176–185. https://doi.org/10.1161/01.RES.0000200162.94463.d7

Tzima, E., Irani-Tehrani, M., Kiosses, W. B., Dejana, E., Schultz, D. A., Engelhardt, B., … Schwartz, M. A. (2005). A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature*, *437*(7057), 426–431. https://doi.org/10.1038/nature03952

Tzima, E., del Pozo, M. A., Shattil, S. J., Chien, S., & Schwartz, M. A. (2001). Activation of integrins in endothelial cells by fluid shear stress mediates Rho-dependent cytoskeletal alignment. *The EMBO Journal*, *20*(17), 4639–4647. https://doi.org/10.1093/emboj/20.17.4639

Wang, Z., & Thurmond, D. C. (2010). Differential phosphorylation of RhoGDI mediates the distinct cycling of Cdc42 and Rac1 to regulate second-phase insulin secretion. *Journal of Biological Chemistry*, *285*(9), 6186–6197. https://doi.org/10.1074/jbc.M109.072421

Whalley, H. J., Porter, A. P., Diamantopoulou, Z., White, G. R., Castaneda-Saucedo, E., & Malliri, A. (2015). Cdk1 phosphorylates the Rac activator Tiam1 to activate centrosomal Pak and promote mitotic

spindle formation. *Nature Communications*, *6*, 7437. https://doi.org/10.1038/ncomms8437

Woods, B., Lai, H., Wu, C. F., Zyla, T. R., Savage, N. S., & Lew, D. J. (2016). Parallel actin-independent recycling pathways polarize Cdc42 in budding yeast. *Current Biology*, *26*(16), 2114–2126. https://doi.org/10.1016/j.cub.2016.06.047

Wu, J., Song, M., Li, T., Zhu, Z., & Pan, J. (2015). The Rho-mDia1 signaling pathway is required for cyclic strain-induced cytoskeletal rearrangement of human periodontal ligament cells. *Experimental Cell Research*, *337*(1), 28–36. https://doi.org/10.1016/j.yexcr.2015.07.016

Xiao, Y., Lin, V. Y., Ke, S., Lin, G. E., Lin, F. T., & Lin, W. C. (2014). 14-3-3Tau promotes breast cancer invasion and metastasis by inhibiting RhoGDIalpha. *Molecular and Cellular Biology*, *34*(14), 2635–2649. https://doi.org/10.1128/MCB.00076-14

Xie, F., Shao, S., Aziz, A. U. R., Zhang, B., Wang, H., & Liu, B. (2017). Role of Rho-specific guanine nucleotide dissociation inhibitor alpha regulation in cell migration. *Acta Histochemica*, *119*(3), 183–189. https://doi.org/10.1016/j.acthis.2017.01.008

Zaidel-Bar, R., Kam, Z., & Geiger, B. (2005). Polarized downregulation of the paxillin-p130CAS-Rac1 pathway induced by shear flow. *Journal of Cell Science*, *118*(Pt 17), 3997–4007. https://doi.org/10.1242/jcs.02523

Zhang, B., Xie, F., Aziz, A. U. R., Shao, S., Li, W., Deng, S., … Liu, B. (2019). Heat shock protein 27 phosphorylation regulates tumor cell migration under shear stress. *Biomolecules*, *9*(2), 50. https://doi.org/10.3390/biom9020050

How to cite this article: Xie F, Shao S, Zhang B, et al. Differential phosphorylation regulates the shear stress-induced polar activity of Rho-specific guanine nucleotide dissociation inhibitor α. *J Cell Physiol*. 2020;1–12. https://doi.org/10.1002/jcp.29594