主論文の要旨

S100A10 is required for the organization of actin stress fibers and promotion of cell spreading

（S100A10 はアクチンストレスファイバーの形成と細胞の伸展に必要である）

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Abstract

Dynamic remodeling of the actin cytoskeleton is crucial for biological processes such as cell migration and cell spreading. S100A10 is a member of S100 protein family and is involved in intracellular trafficking and cell migration. In this study, we examined the role of S100A10 in actin cytoskeleton organization and cell spreading. Depletion of S100A10 induced disruption of stress fiber formation and delay in cell spreading. Rac1 activation was suppressed by S100A10 knockdown, and exogenous expression of active Rac1 restored the ability of cells to spread in the absence of S100A10. Our results demonstrate the crucial role of S100A10 in actin dynamics promoting cell spreading via Rac1 activation.

Results

In order to investigate the role of S100A10 in actin cytoskeleton organization, we used two different siRNAs to deplete S100A10 in cells, HeLa cells were transfected with control or S100A10 siRNAs, and 72 h later the cells were lysed and expression of S100A10 was determined, both siRNAs targeting S100A10 efficiently suppressed its expression(Fig.1a) interestingly, S100A10 knockdown cell became more round compared to control siRNA transfected cells (Fig. 1b), using these siRNAs, we examined the effects of S100A10 depletion on actin cytoskeleton organization. Cells cultured on fibronectin-coated glass cover slips were transfected with either control or S100A10 siRNAs, and 72h later were fixed and immunostained for F-actin using rhodamine conjugated phalloidin, actin stress fiber organization was found to be disrupted in the S100A10 knock down cells (Fig. 1c). These results suggest that S100A10 is required for the organization of stress fibers and the formation of focal adhesions. Next we determined whether S100A10 knockdown diminished the activity of RhoA, a small GTPase essential for stress fiber formation. HeLa cells transfected with control or S100A10 siRNA were lysed and affinity precipitated with GST-Rhotekin-RBD to determine the amount of active RhoA in cells as shown in Fig. 1d, the activity of RhoA was not suppressed by the depletion of S100A10.

Dynamic remodeling of actin organization is crucial for the promotion of cell spreading. We tested whether cell spreading was delayed in the absence of S100A10. HeLa cells transfected with siRNAs were resuspended in serum-free medium and seeded onto a fibronectin-coated surface. At 30 or 60 min post-seeding, the cells were fixed, and the ratio of spread cells was evaluated. Approximately 50% of control siRNA-transfected cells were spread 30 min after seeding, whereas only 15% of the S100A10 depleted cells showed spread morphology (Fig. 2). A significant difference in the ratio of spread cells between control and S100A10 siRNA-transfected cells was also observed 60 min after seeding (Fig. 2), these results suggest that S100A10 is required for the promotion of cell spreading.

Several studies reported that S100A10 is localized at the cytoplasm around nucleus. We inserted S100A10 gene into a GFP plasmid vector. Both HeLa and MDA-MB-231 cells were

transfected with the S100A10-GFP vector, transfected cells were resuspended and seeded on fibronectin coated coverslip, 30 and 60 minutes after post seeding pictures were taken to determine the localization of S100A10 during spreading. Fig.3 shows that S100A10 is localized at the edge of the cell membrane at 30 min of post seeding but not significant after 60 min of post seeding, which indicate that S100A10 is involved in the lamellipodia formation during initial cell spreading

Previous studies have shown that activation of the small GTPase, Rac1, is essential for the promotion of cell spreading. We determined the activity of the Rac1 in control and S100A10 siRNA-transfected cells during spreading. siRNA-transfected cells were resuspended and seeded onto fibronectin coated surface, and 30 min later the cells were lysed and affinity precipitated with GST-PAK-PBD bound to glutathione beads. In this assay, the active form of Rac1 is preferentially affinity precipitated. During cell spreading, the amount of active Rac1 was significantly lower in S100A10-knockdown cells compared to control cells (Fig. 4a). We next tested whether exogenous expression of active Rac1 could overcome the spreading defect induced by S100A10 depletion. HeLa cells constitutively expressing either wild type (WT-Rac) or active rac (Ac-Rac) were generated by retrovirus infection. Results from the pull-down assay clearly showed an increase in active Rac1 levels in Ac-Rac cells compared to WT-Rac cells (Fig. 4b). These cells were transfected with either control or S100A10 siRNAs, and cell spreading was evaluated 72 h later as shown in Fig. 4c, the expression of active Rac1 significantly restored ability of cells to spread, these results indicate that S100A10 controls cell spreading via Rac1 activation.

In order to further confirm requirement of S100A10 for cell spreading , we used another cell line, MDA-MB-231, in our assays, as observed in HeLa cells, silencing of S100A10 induced the disruption of stress fiber and focal adhesion formation in MDA-MB-231 cells (Fig. 5a). We also examined cell spreading in the absence of S100A10 as shown in Fig. 5b, cell spreading was significantly delayed upon S100A10 knockdown. In addition, Rac1 activation during cell spreading was suppressed by S100A10 depletion (Fig. 5c). These results further confirm the crucial role of S100A10 in actin cytoskeleton organization and cell spreading.

Discussion

In this study, we showed that depletion of S100A10 using two different siRNAs induced the disruption of actin stress fiber and focal adhesion formation in HeLa and MDA-MB-231 cells. Actin organization was regulated by diverse set of signaling proteins. One of the major players is a small GTPase RhoA. Activated RhoA promotes the activation of downstream effectors such as ROCK and induces actin filament formation. We tested whether the disruption of stress fiber formation by S100A10 depletion was mediated by RhoA inactivation, but did not observe any decrease of RhoA activity in the absence of S100A10 expression. For the organization of actin stress fibers, S100A10 may be required for the activation of signaling

pathway downstream of RhoA. Alternatively, S100A10 may regulate stress fiber formation independent of RhoA. Stress fibers are composed of 10 to 30 actin filaments, and numerous actin binding proteins are required for actin bundling. Previously, ANX2 has been shown to be an actin-binding protein that is critical for the regulation of actin dynamics. Although further analysis is necessary, it is reasonable to hypothesize that S100A10 is required for ANX2 to maintain actin stress fiber organization.

We found that S100A10 is involved in the process of cell spreading. In the absence of S100A10, cell spreading was delayed in both HeLa and MDA-MB-231 cells. Upon cell adhesion to the extracellular matrix, a number of signaling pathways are triggered to activate Rac1 for the induction of actin reorganization at the periphery of cells to promote membrane extension. We observed that activation of Rac1 during cell spreading was suppressed in the absence of S100A10. In addition, the expression of active Rac1 restored the ability of cells to spread. These results suggest that delayed cell spreading by S100A10 knockdown is mediated by the suppression of Rac1 activation. It has been demonstrated previously that S100A10 depletion suppresses cell migration. Activation of Rac1 is crucial for the membrane extension to promote migration. Since common regulatory mechanisms are utilized in the generation of membrane protrusions during cell spreading and migration, it is possible that S100A10 may also be required for Rac1 activation during cell migration.