博士論文（要約）

Functional roles of Rho-GEF Solo in regulation of actin and intermediate filament networks and mechanotransduction

（Rho-GEF Solo によるアクチン繊維と中間径フィラメントの制御とメカノセンシングにおける機能）

平成 27 年度

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All the cells in our body are exposed to mechanical forces. The mechanical force-induced cytoskeletal reorganization is essential for numerous pathophysiological processes, such as tissue morphogenesis and homeostasis. Mechanotransduction is a process that cells respond to external forces by converting mechanical force signals to biochemical signals. Epithelial cells perceive external forces primarily through cell-cell and cell-substrate adhesion sites, resulting in reinforcement of actin and intermediate filament (IF) networks. Rho family GTPases are activated by Rho-guanine nucleotide exchange factors (Rho-GEFs) and essential for actin reorganization. However, the mechanisms underlying the regulation of force-induced Rho activation remain elusive.

Cyclic stretch is an artificial model of mechanical force loading, which induces the reorientation of vascular endothelial cells (ECs) and their actin stress fibers in a direction perpendicular to the stretch axis. Abiko et al. conducted a screen of short hairpin RNAs targeting 63 Rho-GEFs and demonstrated that at least 11 Rho-GEFs (Abr, Alsin, ARHGEF10, Bcr, GEF-H1, LARG, p190RhoGEF, PLEKHG1, P-REX2, Solo and α-PIX) are involved in the stretch-induced perpendicular reorientation of ECs. Among these Rho-GEFs, I examined the role of Solo (the GEF for RhoA and RhoC) in cyclic-stretch-induced responses of ECs. Expression of Solo induced RhoA activation and F-actin accumulation at cell-cell and cell-substrate adhesion sites (Figure 1). I showed that knockdown of Solo significantly suppressed cyclic-stretch-induced perpendicular reorientation of ECs, when cells were cultured at drug-free control conditions, but the suppressive effect of Solo knockdown was not detected when cells were pretreated with EGTA or VE-cadherin- targeting siRNAs

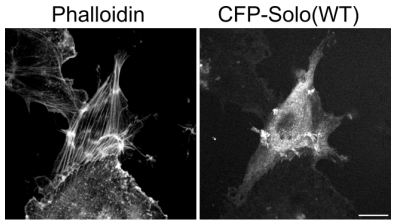

Figure 1. Solo induces F-actin accumulation at cell-cell and cell-substrate adhesion sites in vascular endotherial cells. Scale bar, 20 μm.

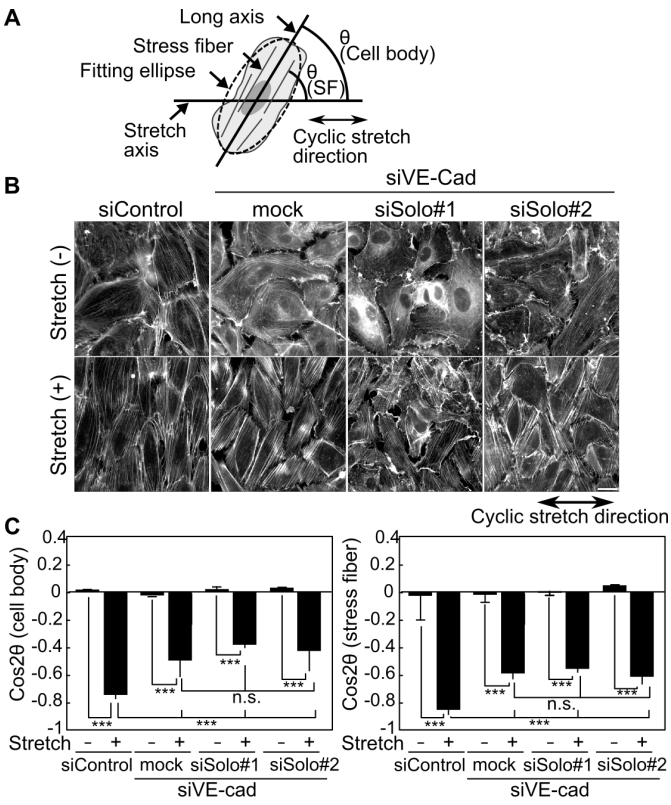

Figure 2. Knockdown of VE-cadherin abrogates the suppressive effect of Solo knockdown on cyclic-stretch-induced cell and SF orientation. (A) Analysis of SF and cell body orientation. The angle (θ) relative to the stretch axis was measured. (B) Effects of VE-cadherin knockdown on cyclic-stretch-induced cell and SF orientation. (C) The orientation parameters (cos2θ) of cell bodies (left) and SFs (right) were measured. The values of cos2θ=0 and -1 indicate the random and perpendicular orientation, respectively. Scale bar, 20 μm.

(Figure 2). I also showed that knockdown of Solo suppressed force-induced RhoA activation by biochemical analyses (Figure 3). These results suggest that Solo is involved in cell-cell-contact- and VE-cadherin-mediated mechanical signal transduction during cyclic-stretch-induced cell and stress fiber reorientation of ECs.

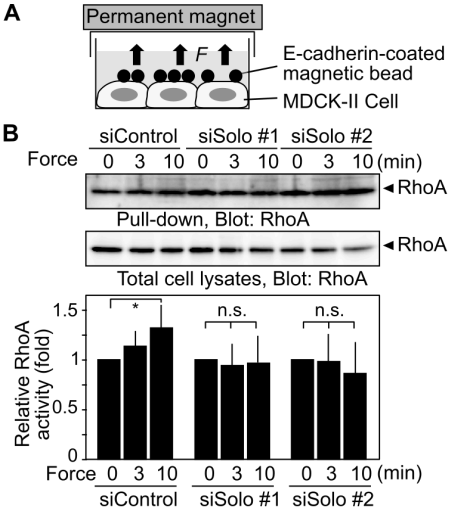

Figure 3. Solo is required for tensile-force-induced RhoA activation. (A) Scheme of the process of tensile force application. (B) Intracellular RhoA activity was analyzed by GST-rhotekin(RBD) pull-down assays. Knockdown of Solo suppresses tensile-force-induced RhoA activation.

IFs are stable but resilient cytoskeletal filaments that provide structural support for cells. Keratins are major IFs in epithelia. I examined the interaction between keratin IFs and Solo. Solo binds to keratins-8/keratin-18 (K8/K18) IFs through multiple sites. Solo overexpression in epithelial cells promoted the formation of thick stress fibers and keratin bundles, whereas knockdown of Solo or expression of a GEF-inactive mutant of Solo suppressed stress fiber formation and led to disorganized keratin networks. To examine the roles of Solo and keratin IFs in mechanotransduction, I developed the time-lapse observation system of tensional-force-induced stress fiber formation. I showed that knockdown of Solo or K18 or overexpression of GEF-inactive or deletion mutants of Solo suppressed tensile-force-induced stress fiber formation. These results suggest that the interplay between Solo and K8/K18 filaments plays a crucial role in tensile-force-induced RhoA activation and consequent actin cytoskeletal reinforcement.