ROCKing Upstream: Rho-Associated Kinase 1 Mediates RhoA Activity through a Novel Feedback Loop

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Actin cytoskeleton reorganization induces morphological changes in mammalian cells. This remodeling process directly affects cell motility, which is a prerequisite for cancer metastasis. The Rho family of small GTPases has been shown to tightly regulate actin cytoskeleton organization and stability. In prostate cancer cells, RhoA is a critical regulator that promotes cell invasion and migration. The inhibition of RhoA and its major downstream effector, Rho-Associated Kinase 1 (ROCK1), has been demonstrated to inhibit prostate cancer cell motility. We previously found that Y-27632 and Fasudil (HA-1077), selective ROCK1 inhibitors, diminished RhoA activity (GTP-RhoA) in prostate cancer (PC3) cells. Based on these results, we hypothesized that ROCK1 participates in a feedback mechanism to regulate upstream RhoA activity. In this study, we used genetic manipulation (siRNA knockdown) in conjunction with a pharmacological approach in order to elucidate that the ROCK1 mediation of RhoA occurs through the inhibitory effect of ROCK1 upon a Rac1 guanine nucleotide exchange factor (GEF), TIAM1. Inhibition of ROCK1 markedly increased Rac1 activity but reduced RhoA activity. Previous studies have demonstrated that Rac1 is able to antagonize RhoA activity through a redox-dependent mechanism. Accordingly, the increased levels of active Rac1 would explain the decreased levels of active RhoA. Additional experiments showed that direct inhibition of Rac1 increased RhoA activity. Furthermore, the siRNA knockdown of Rac1 severely diminished the inhibitory effect of Fasudil on RhoA activity. Upon knockdown of TIAM1, the ability of Fasudil to increase Rac1 activity and decrease RhoA activity was abolished. Taken together, these findings indicate for the first time that ROCK1 regulates upstream RhoA activity by mediating levels of active Rac1. The ability of ROCK1 to regulate upstream Rho GTPases through signaling pathway crosstalk introduces novel approaches to drug development for the treatment of metastatic prostate cancers and various other pathological conditions.