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Rictor encounters RhoGDI2

The second pilot is taking a lead

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Rictor's role in cell migration has been first indicated in the original chemotaxis studies in *Dictyostelium* and more recent studies reported that rictor is required for migration of cancer cells. How rictor promotes cell migration remains poorly characterized. Based on our proteomics study we have identified a novel functional role of rictor in regulation of cell migration. Here, we discuss our recent finding that rictor by suppressing RhoGDI2 maintains activity of the Rac1/cdc42 GTPases and promotes cell migration. Our finding outlines a critical role of rictor in the regulation of RhoGDI2 activity. This study opens new avenues in the investigation of cancer metastasis by analyzing the rictor dependent post-translational modification of RhoGDI2.

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Directed cell migration is a fundamental cellular response required for embryonic development, inflammatory response and wound repair.[1] The Rho GTPases provide major role in coordinating the cellular responses required for cell migration. Rho proteins act as molecular switches cycling between an active GTP-bound and inactive GDP-bound conformations. This activity is tightly controlled by the guanine nucleotide exchange factors and GTPase activating proteins that determine a ratio of active or inactive Rho GTPases in cells.[2] Rho specific guanine nucleotide dissociation inhibitors (RhoGDIs) represent additional class of regulatory proteins that are critical to control activity of Rho GTPases. By forming heterodimers with the inactive GDP-bound Rho GTPases, the RhoGDI proteins control the Rho GTPase partitioning between the cytosol and membrane compartments.[3]

In mammals, the RhoGDI family is represented by three members, each member displays a specific pattern of tissue distribution and activity toward Rho GTPases.[2] RhoGDI1, as the most abundant and ubiquitously expressed member of the RhoGDI family, has been actively studied and characterized.[2] Similar to RhoGDI1, the second member RhoGDI2 is also a conserved member of this family and plays an important role in cell migration.[4-10] The third member of this family RhoGDI3 is a least conserved member of RhoGDIs and known to be expressed preferentially in brain, pancreas, lung, kidney and testis.[11]

The initial characterization of RhoGDI2 identified its tissue specific expression in the blood forming cells, but the later study determined its expression in a wide range of cell types.[5] RhoGDI2 along with RhoGDI1 share common structural features and interact with the same and distinct Rho GTPases to regulate their membrane association.[12] Despite a high degree of structure similarity, some studies suggest that both RhoGDIs have specific binding affinities toward active Rho GTPases.[13-15] RhoGDI1 preferentially binds and inhibits RhoA,[13,15] whereas RhoGDI2 has been shown to bind and inhibit Rac GTPase.[14] Remarkably, RhoGDI2 has been identified as a marker lost in bladder cancer metastasis and its loss correlates with a decreased patient

survival.[5] Similarly, others have reported that abundance of RhoGDI2 is either decreased or lost in several invasive cancers.[4,7,9] Given the increasing evidence regarding a loss of RhoGDI2 in cancer metastasis, it is important to explore the molecular mechanism of regulation of RhoGDI2 and its implication in cancer cell migration and metastasis. Our recent study presented in *Oncogene* reveals that rictor (rapamycin-insensitive companion of mTOR) controls the functional activity of RhoGDI2.[16]

Initially, the Devreotes group has identified the rictor's ortholog in Dictyostelium as a regulator of chemotaxis.[17] Later, the biochemical studies of the mammalian Target of Rapamycin (mTOR), the essential protein kinase known as a central component of the highly conserved nutrient-sensing pathway, defined rictor as the mTOR interacting protein.[18,19] Binding of rictor and Sin1 to mTOR forms mTORC2 (the mTOR Complex 2) distinct from the complex assembled by mTOR and raptor (mTORC1). mTORC1 is a nutrient-sensitive complex that regulates protein synthesis by phosphorylating the regulators of protein synthesis S6K1 and 4EBP1.[20] The second mTOR complex, mTORC2 functions as the regulatory kinase of the distinct members of AGC kinase family including Akt, SGK and PKCα. In yeast and mammalian cells, mTORC2 has been shown to regulate cell morphology, at least in part, by controlling protein kinase Cα[18] and activity of the Rho GTPases.[19,21]

The functional studies of rictor have been mostly focused on its role as the essential component of mTORC2. The recent studies are also beginning to shed the light on a role of rictor in cell migration independent of mTORC2. It has been shown that rictor alone without its binding partner Sin1 interacts with the regulators of cell morphology and migration known as the integrin-linked kinase and actin-based molecular motor myosin-1c.[22,23] In our recent work, we found that a loss of rictor leads to induction of RhoGDI2 and interferes with cell migration by inhibition of Rac1 and cdc42 GTPase activity. Importantly, the rictor binding protein Sin1, an integral component of mTORC2, has not been linked to the regulation of RhoGDI2 indicating a novel mTORC2 independent function of rictor in cell migration.[16]

To address a functional role of rictor in regulation of cell migration, we performed an unbiased proteomic analysis of the rictor null mouse embryonic fibroblasts (MEFs). We identified several differentially expressed proteins correspond to actin filament assembly and stress response pathways.[16] Cofilin and destrin were shown to play an important role in the assembly of actin filament by forming new polymerization-competent filament barbed ends through their severing activity[24] were low expressed proteins in the rictor null cells. These differences might reflect a lower cytoskeleton dynamics of the elongated rictor null cells.[16] On the contrary, we also observed a high abundance of RhoGDI2 in the rictor null cells,[16] which is known to function as an important negative regulator of Rho GTPases.[2]

RhoGDI2 is highly conserved protein containing two structurally distinct regions. The N-terminal flexible region (residues 1–74) defines its "regulatory arm" and is responsible for the GTPase inhibitory function through interactions with the Rac GTPase, whereas the C-terminal region (residues 75–204) accommodates the isoprenyl moiety of the Rac GTPase in its hydrophobic pocket, and is required to extract Rac GTPases from the membrane (**Fig. 1A**). Therefore, both regions of RhoGDI2 contribute significantly in the Rac binding and the GTPase activity of the Rac.[14] Our finding is consistent with the previous studies indicating that RhoGDI2 interacts with Rac1 and Cdc42 but with a higher affinity toward Rac1 carrying a substantial inhibitory effect on GTPase activity of Rac1.[16] Thus, the association of RhoGDI2-Rac1 GTPase complex is likely plays an important role in the regulation of Rac1 GTPase activity. A key question remains to be answered is how rictor regulates RhoGDI2. This regulation is not related to the gene expression mechanism because overexpression of the wild type RhoGDI2 does not mimic the effect of a loss of rictor in the wild type MEFs, although a suppression of RhoGDI2 in the rictor null MEFs is effective in rescue of cell migration.[16] It is likely to be mediated by a rictor dependent post-translational modification of RhoGDI2 at the extreme N-terminal regulatory sequence.

The importance of the functional role of the N-terminal region of RhoGDI2 has been previously described with truncated N-terminally proteins.[25] Deletion of the first 41 amino acid residues from RhoGDI2 resulted in complete loss of its ability to bind Rac1 and GTPase activity.[25] Although the folded regions of the RhoGDI1 and RhoGDI2 show 74% sequence identity, whereas the N-terminal sequence, with the first 25 amino acid residues show only 24% of identity and the fragment representing residues between amino acids 26–74 carries identity of 54.5% (**Fig. 1A**). Therefore, we assume that rictor alone forms a distinct complex with the unknown kinase and regulate the phosphorylation in the extreme N-terminal amino acid residues that varies between RhoGDI1 and RhoGDI2 (**Fig. 1B**). Phosphorylation of RhoGDIs has emerged as one of the key post-translational modifications that may play a major role in the dissociation of the cytosolic RhoGDIs-GTPase complexes.[26-29] Several phosphorylation sites have been identified on RhoGDI1 such as Ser101 and Ser174 phosphorylation by p-21-activated kinase[30] and Ser34 by protein kinase Cα (PKCα)[28] promoting the release of RhoA and Rac1 respectively. Recently, similar phosphorylation site (Ser31) by PKCα was identified on RhoGDI2.[29] Griner and his colleagues reported that PKCα-mediated phosphorylation of Ser31 destabilizes the N-terminus of the RhoGDI2 that interacts mainly with the switch regions of Rac1 GTPases most likely accounting for the decrease in RhoGDI2 binding to Rac1 and promotes its GTPase activity.[29] Therefore it is also possible that the functional activity of RhoGDI2 controlled by rictor depends on other type of the post-translational modifications (the extreme N-terminal regulatory region) such as acetylation or ubiquitilation/sumoylation (**Fig. 1B**) because the RhoGDI1 sumoylation at Lys-138 has been shown to be crucial for cell migration.[31]

RhoGDI2 has been actively studied in cancer metastasis and the expression levels

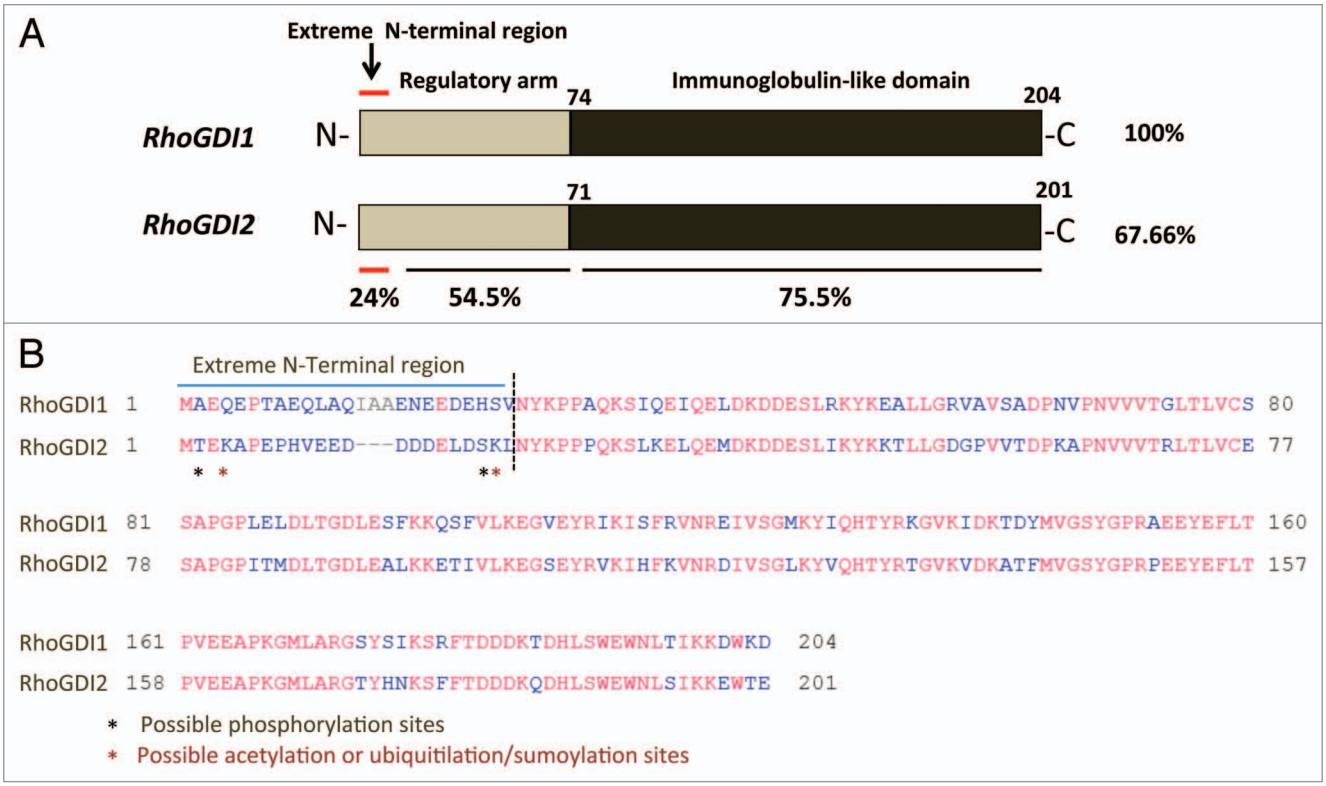

Figure 1. (**A**) Structure of RhoGDI1 and RhoGDI2. Primary sequence similiarity of RhoGDI1 and RhoGDI2 as indicated by the percent identity. The approximate locations of important residues described in the text are indicated the terminal amino acid residue. First 25 amino acid residues are defined as extreme N-terminal region. (**B**) Sequence alignments of human RhoGDI1 and RhoGDI2 were performed using COBALT FORMAT. *Indicated the possible post-translational modication residues.

of RhoGDI2 are severely altered in a wide range of cancers.[4-10] It has been reported that the abundance of RhoGDI2 is either decreased or lost in several cancers.[4,5,7,9,10] In contrary, the studies in pancreatic cancer reported a high abundance of RhoGDI2 and it correlates with increased invasiveness.[8] In breast cancer, the pattern of expression of RhoGDI2 is biphasic. There is evidence that the expression of RhoGDI2 is increased at the early stages of cancer progression but decreased sharply during metastasis.[6] We believe that these studies by focusing on detection of the RhoGDI2 abundance as a marker do not reflect the functional activity of RhoGDI2. Most likely, the functional role of RhoGDI2 as a potent inhibitor of cell migration is dependent on its activity regulated by phosphorylation or other posttranslational modifications within extreme N-terminal domain because it is distinct from RhoGDI1. Overall, our study points out on the potent rictor dependent regulation of the RhoGDI2 functional activity and understanding of this mechanism carries a promise to translate into the novel approach to control cell migration and treat cancer metastasis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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