Functional Characterization of a Novel RhoGAP Protein Deleted in Liver Cancer 2 (DLC2)

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Deleted in liver cancer 2 (DLC2) is a candidate tumor suppressor protein found to be underexpressed in liver samples of patients with hepatocellular carcinoma (HCC). Here we report on the functional characterization of DLC2. DLC2 protein contains three domains, which are sterile α motif (SAM), Rho GTPase-activating protein domain (GAP) and steroidogenic acute regulatory protein-related lipid transfer domain (START). Database searching and RT-PCR analyses revealed that there are four isoforms of DLC2, namely α, β, γ and δ. In vitro and in vivo studies confirmed that the GAP and START domains in DLC2 are functional. In vitro GAP assay indicated that DLC2 GAP preferentially activated hydrolysis of GTP in both Rho and Cdc42 GTPases, but not Rac GTPase. Overexpression of DLC2 in HeLa and Huh-7 cell lines induced the depolymerization of stress fiber, leading to morphological changes and remodeling of actin cytoskeleton. Deletion studies demonstrated that the GAP domain alone is responsible for actin depolymerization, and neither SAM nor START was involved in that process, although those two domains might attenuate the function of GAP. For the study of START domain, we overexpressed either a myc-tagged or V5-tagged form of DLC2 START domain in Huh-7 hepatoma cells. Those two mutants were dispersedly distributed to peculiar subcellular compartments around lipid droplets in the cytosol. We also assessed the involvement of DLC2 START domain in the lipid transport/efflux in hepatoma cells, and we found that either full-length DLC2 or truncated DLC2 mutant with the START domain could only up-regulate the expression of ATP-binding cassette transporter ABCA1, an important mediator of phospholipid and cholesterol efflux. Taken together, our findings suggest that DLC2 can remodel cell morphology through its GAP domain and modulate cellular lipid transfer through its START domain.