GoMADScan

RAS is the founding member of a superfamily of GTPases and regulates signaling pathways involved in cellular growth control. While recent studies have shown that the activation state of RAS can be controlled by lysine ubiquitylation and acetylation, the existence of lysine methylation of the RAS superfamily GTPases remains unexplored. In contrast to acetylation, methylation does not alter the side chain charge and it has been challenging to deduce its impact on protein structure by conventional amino acid substitutions. Herein, we investigate lysine methylation on RAS and RAS-related GTPases. We developed GoMADScan (Go language-based Modification Associated Database Scanner), a new user-friendly application that scans and extracts posttranslationally modified peptides from databases. The GoMADScan search on PhosphoSitePlus databases identified methylation of conserved lysine residues in the core GTPase domain of RAS superfamily GTPases, including residues corresponding to RAS Lys-5, Lys-16, and Lys-117. To follow up on these observations, we immunoprecipitated endogenous RAS from HEK293T cells, conducted mass spectrometric analysis and found that RAS residues, Lys-5 and Lys-147, undergo dimethylation and monomethylation, respectively. Since mutations of Lys-5 have been found in cancers and RASopathies, we set up molecular dynamics (MD) simulations to assess the putative impact of Lys-5 dimethylation on RAS structure. Results from our MD analyses predict that dimethylation of Lys-5 does not significantly alter RAS conformation, suggesting that Lys-5 methylation may alter existing protein interactions or create a docking site to foster new interactions. Taken together, our findings uncover the existence of lysine methylation as a novel posttranslational modification associated with RAS and the RAS superfamily GTPases, and putative impact of Lys-5 dimethylation on RAS structure [1)].

1)

Yoshino H, Yin G, Kawaguchi R, Popov KI, Temple B, Sasaki M, Kofuji S, Wolfe K, Kofuji K, Okumura K, Randhawa J, Malhotra A, Majd N, Ikeda Y, Shimada H, Kahoud ER, Haviv S, Iwase S, Asara JM, Campbell SL, Sasaki AT. Identification of lysine methylation in the core GTPase domain by GoMADScan. PLoS One. 2019 Aug 7;14(8):e0219436. doi: 10.1371/journal.pone.0219436. eCollection 2019. PubMed PMID: 31390367.