ARHGAP21 MODULATES FAK ACTIVITY AND DOWNSTREAM EFFECTORS IN T98G GLIOMA CELL LINE

Bigarella C.L., Borges L.,SallesT.S.I.,Saad S.T.O.

Hemocentro e Departamento de Clínica Médica, FCM, UNICAMP, São Paulo, Brazil

ARHGAP21 is a RhoGAP with GAP activity against Cdc42 and RhoA. ARHGAP21 is known to interact with active ARF-GTPases, regulating actin dynamics on Golgi membranes, and also with alpha-catenin on endothelial sites of bacterial invasion. The aim of our work was to investigate the involvement of ARHGAP21 on FAK stimulated signaling pathways in T98G glioma cells by ARHGAP21 knockdown. Co-immunoprecipitation assays showed association of ARHGAP21 with FAK in T98G cells. Pull down assays using GST fusion proteins consisting of Ferm, Catalytic and C-terminal regions of FAK protein confirmed association of ARHGAP21 to the C-terminal region of FAK. T98G transfected with a shRNAi to knockdown Arhgap21 expressed at least 50% less ARHGAP21 ($T98G^{-Arhgap21}$ cells). The level of total FAK protein has not been altered by ARHGAP21 depletion but its phosphorylated counterparts have been up-regulated. FAK phosphorylated on Tyr397 and Tyr 925 showed 3 to 14 folds and 2 to 3 folds increases, respectively. The downstream signaling molecules Src and $p130^{Cas}$ showed also increased phosphorylation on $T98G^{-Arhgap21}$, 2 to 13 folds and 2 to 4 folds, respectively. Migration assays displayed an increase of 20 to 50% on migration rates of $T98G^{-Arhgap21}$, indicating that the signaling pathways altered by ARHGAP21 depletion leads to increased migration rates.These results evidenced the involvement of ARHGAP21 modulating FAK activity and its downstream signaling pathways orchestrating T98G cells mobility.