Hyaluronan-CD44 interaction with leukemia-associated RhoGEF and epidermal growth factor receptor promotes Rho/Ras co-activation, phospholipase C epsilon-Ca2+ signaling, and cytoskeleton modification in head and neck squamous cell carcinoma cells.

Abstract:
In this study we have examined the interaction of CD44 (a major hyaluronan (HA) receptor) with a RhoA-specific guanine nucleotide exchange factor (leukemia-associated RhoGEF (LARG)) in human head and neck squamous carcinoma cells (HNSCC-HSC-3 cell line). Immunoprecipitation and immunoblot analyses indicate that CD44 and the LARG protein are expressed in HSC-3 cells and that these two proteins are physically associated as a complex. HA-CD44 binding induces LARG-specific RhoA signaling and phospholipase C epsilon (PLC epsilon) activity. In particular, the activation of RhoA-PLC epsilon by HA stimulates inositol 1,4,5-triphosphate production, intracellular Ca2+ mobilization, and the up-regulation of Ca2+/calmodulin-dependent kinase II (CaMKII), leading to phosphorylation of the cytoskeletal protein, filamin. The phosphorylation of filamin reduces its interaction with filamentous actin, promoting tumor cell migration. The CD44-LARG complex also interacts with the EGF receptor (EGFR). Most importantly, the binding of HA to the CD44-LARG-EGFR complex activates the EGFR receptor kinase, which in turn promotes Ras-mediated stimulation of a downstream kinase cascade including the Raf-1 and ERK pathways leading to HNSCC cell...
growth. Using a recombinant fragment of LARG (the LARG-PDZ domain) and a binding assay, we have determined that the LARG-PDZ domain serves as a direct linker between CD44 and EGFR. Transfection of the HSC-3 cells with LARG-PDZcDNA significantly reduces LARG association with CD44 and EGFR. Overexpression of the LARG-PDZ domain also functions as a dominant-negative mutant (similar to the PLC/Ca2+-calmodulin-dependent kinase II (CaMKII) and EGFR/MAPK inhibitor effects) to block HA/CD44-mediated signaling events (e.g. EGFR kinase activation, Ras/RhoA co-activation, Raf-ERK signaling, PLC epsilon-mediated inositol 1,4,5-triphosphate production, intracellular Ca2+ mobilization, CaMKII activity, filamin phosphorylation, and filamin-actin binding) and to abrogate tumor cell growth/migration. Taken together, our findings suggest that CD44 interaction with LARG and EGFR plays a pivotal role in Rho/Ras co-activation, PLC epsilon-Ca2+ signaling, and Raf/ERK up-regulation required for CaMKII-mediated cytoskeleton function and in head and neck squamous cell carcinoma progression.