SNX 9 INTERACTS WITH DYNAMIN 2 AND N-WASP TO REGULATE FOCAL ADHESION DISASSEMBLY AND CELL MIGRATION

A. Zhubanchaliyev[1], W. T. Chao[2], J. Kunz[1,2*]

1) School of Science and Technology, Nazarbayev University, Astana, Kazakhstan; *Jeanette.Kunz@nu.edu.kz; 2) Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, USA

Introduction. The turnover of focal adhesions is essential for normal cell migration and tumor invasion, but the underlying mechanisms are poorly understood. We have previously shown that the disassembly of focal adhesions occurs by endocytosis of activated integrins from adhesion sites (1). We have further identified new components that mediate integrin endocytosis. Here, we provide the initial characterization of one of these components, sorting nexin 9 (Snx9), an endocytic adaptor protein not previously implicated in cell adhesion and migration.

Materials and methods. To examine the molecular mechanisms underlying Snx9 regulation of focal adhesion disassembly we used a combination of cell biological, biochemical and knockdown approaches in the osteosarcoma cancer cell line HT1080.

Results and discussion. We report here a novel function of Snx9 in cell motility control via regulation of focal adhesion disassembly. Knockdown of Snx9 with short interfering RNA caused a severe defect in focal adhesion disassembly, which resulted from the inhibition of dynamin 2-dependent beta1 integrin endocytosis, thus leading to surface accumulation of integrins and preventing the turnover of focal adhesion complexes. We further show that Snx9 is necessary for the recruitment of dynamin 2 to focal adhesions before adhesion turnover. Snx9 also recruits N-WASP and the actin nucleation complex ARP2/3 to adhesion sites suggesting that Snx9 thereby stimulates actin assembly at focal adhesions to drive integrin endocytosis. Snx9 localization to focal adhesions, in turn, is dependent on $PI4,5P_2$ synthesis by PIPK1p. Notably, Snx9 is known to form a complex with PIPK1P and the tyrosine kinase Activated CDC42 kinase 1 (Ack1) (2,3) and formation of this complex has been shown to stimulate PIPKIp activity and increase $PI4,5P_2$ synthesis (3). These data therefore suggest that Ack1 acts on PIPK1P to locally upregulate $PI4,5P_2$ synthesis for integrin endocytosis and focal adhesion turnover. Consistent with such an idea, we find that Ack1 kinase activity is necessary for focal adhesion disassembly. Furthermore, we identify a potential phospho-tyrosine residue within PIPK1P necessary for focal adhesion disassembly.

Conclusions. Together, these findings suggest that the Snx9-dependent disassembly of focal adhesions is a novel function of Snx9 that is important for cell migration. The results further indicate that Snx9 engages in a positive feedback loop with PIPK1P and Ack1 to drive adhesion disassembly. Notably, Ack1 is a novel anti-cancer target that is overexpressed in breast and prostate cancers and correlated with metastasis and poor patient prognosis (4). This work may thus provide a new principle for understanding the role of Ack1 in cancer metastasis and identifies PIPK1P as an alternative therapeutic target.

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