

A Scaffold Protein in the c-Jun N-terminal Kinase Signaling Pathway is Associated with Focal Adhesion Kinase and Tyrosine Phosphorylated

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Cell adhesion to the extracellular matrix (ECM) regulates many cellular functions, including differentiation, cell growth, apoptosis, and cell migration. Focal adhesion kinase (FAK) is a widely expressed nonreceptor protein tyrosine kinase localized in focal adhesions, and is critical for integrin-mediated signal transduction pathways. FAK becomes activated and tyrosine phosphorylated in response to cell adhesion to ECM proteins. Activated FAK undergoes autophosphorylation at Tyr-397 and thereby binds to the Src homology 2 (SH2) domain of the Src-family kinase, and SH3 domain of phosphatidylinositol 3-kinase (PI 3-K) p85 regulatory subunit. Src phosphorylates FAK and a number of FAK-associated proteins, including p130 Crk-associate substrate (p130^{cas}) and paxillin, which contain docking sites for CrkII SH2 domain. Integrin-mediated activation of c-Jun N-terminal kinase (JNK) requires the association of FAK with Src and p130^{cas}. FAK is also known to promote cell migration by coupling with p130^{cas} and CrkII. A role of JNK activation in cell migration is still controversial, however, several lines of evidence revealed that MEKK1 and activated JNK are localized in focal adhesion. JNK/stress-activated protein kinase-associated protein 1 (JSAP1), the newly identified scaffolding protein for JNK, binds to JNK, MEKK1 and SEK1. JSAP1 functions as a not only scaffolding factor in the JNK cascade but also suppressor in the ERK cascade by binding to Raf-1 and MEK1.

We have demonstrated that JSAP1 forms complex with N-terminus of FAK. The complex formation was further stimulated by c-Src but not by kinase-deficient Src, in which JSAP1 was tyrosine-phosphorylated and other FAK/Src signaling molecules were recruited. The stimulation of JSAP1 binding to FAK by c-Src required Tyr-397 of FAK. The mutant lacking amino acid residues 343-743 of JSAP1, in which tyrosine residues to be phosphorylated are included, effectively bound to FAK without c-Src. These results suggest that the domain within amino acid residues 343-743 of JSAP1 suppresses association with FAK, and phosphorylation of tyrosine in this region releases the suppression. Fibronectin (FN) stimulation of cells expressing JSAP1 induced its tyrosine phosphorylation concomitant with association with FAK. Expression of JSAP1 in HeLa cells facilitated formation of well-organized focal contacts and actin-stress fibers, and promoted cell spreading onto FN. JSAP1-induced cell spreading on FN was suppressed by expression of dominant negative form of JSAP1. Taken together, these results suggest that JSAP1 is involved in integrin-mediated signaling pathway through FAK/Src by recruiting other signaling molecules, resulting in promotion of cell spreading onto FN.