Identification and Characterization of a Novel Cooperative Activity between the Proto-oncogenic SFKs and Crk/CrkL Signaling Protein Families.

Miranda M. Redmond[1*], Tyler M. Aten[1*], Shelia Weaver[2] and Bryan A. Ballif[1]

Departments of Biology[1] and Mathematics and Statistics[2], University of Vermont, Burlington, VT, 05405, USA.

[*]Equal contribution.

Signaling mechanisms initiated by the Src-family of tyrosine kinase's (SFK's) are vital for numerous cellular functions including motility, migration and adhesion. However, when SFK's are unregulated they exhibit oncogenic or cancerous properties. It has long been appreciated that multiple signaling molecules act in concert to facilitate normal biological responses and that multiple oncogenes acting in concert can increase tumorigenic potential. SFKs have been shown in some settings to act in concert with another proto-oncogenic protein family, the Crk/CrkL family of adaptor proteins. Independently, SFKs and Crk/CrkL are known to effectuate signaling mechanisms essential in many biological processes. In this study we sought to identify novel cooperative interactions between these two protein families. Using pharmacological inhibitors in cell culture, biochemical manipulations, and quantitative mass spectrometry, we have identified a novel SFK substrate to which Crk/CrkL proteins are recruited, generating a novel signaling scaffold with implications in both normal and aberrant biological activities. Furthermore, through mutational analysis we characterize the specific SFK-dependent phosphorylation sites that induce CrkL binding. The implications of this novel signaling complex are discussed.