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Application of a Targeted Proteomics Approach for the Identification of KSR-associated Proteins

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Kinase suppressor of Ras (KSR) was discovered as a conserved component of the Ras signaling cascade in a genetic screening performed in *Drosophila*. It is thought to act as a protein scaffold for proteins involved in the Ras signal transduction pathway. Experiments have shown that KSR migrates to the plasma membrane upon an activation signal where KSR organizes an enzyme-substrate complex, which facilitates a series of phosphorylation reactions, activating MAPK and other downstream target proteins. However, a number of the protein components in the signaling complex, regulation of KSR translocalization and the detailed translocalization mechanism are largely unknown. Here, we have applied a targeted proteomics approach utilizing mass spectrometry to identify the individual proteins in the KSR signaling complex isolated by immuno-precipitation. These discoveries have profound biological implications for the functions of the KSR signaling complex and further our understanding of the Ras signal transduction pathway.

KSR was expressed in COS cells by recombinant adenovirus. The cells were lysed in 25 mM Tris, pH 7.5 with 0.1% of Triton X-100. Various full length and truncated forms of KSR protein signaling complexes were purified by immunoprecipitation. Immunoprecipitated proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and stained using coomassie blue. An in-gel tryptic digestion was performed on the visible gel bands and the extracted tryptic peptides were analyzed by either matrix associated laser desorption/ionization with a time-of-flight/time-of-flight (TOF/TOF) mass spectrometer (MS) or by reversed-phase microcapillary liquid chromatography (μLC) coupled online to a quadrupole ion-trap (IT) MS. Collision-induced dissociation spectra from either the TOF/TOF or the IT MS were searched against the non-redundant *Homo sapiens* FASTA database.

The MS analyses of the various immunoprecipiated KSR complexes resulted in the identification of a number of functionally different proteins associated with KSR in the signaling complex as illustrated in Figure 1. A group of heat shock and chaperone proteins, such as Hsp90, Cdc37, and TCP, were found to be associated with KSR. Several signaling proteins that have been previously shown to associate with KSR, such as MEK and 14-3-3, were also identified. Several 14-3-3 isoforms previously not known to associate with KSR were also found in the complex, suggesting that KSR may associate with a 14-3-3 hetero-dimer. Another interesting protein, programmed cell death 8 isoform 2, was also found to bind within the KSR complex.

By analyzing different complexes with truncated forms of mutant KSR, the specific KSR binding regions for these interacting proteins were also located. For example, heat shock proteins and chaperones were only found to associate with the CA5 domain of KSR. 14-3-3 proteins and C-TAK1 interacts with N-terminal of KSR. All of the results identified via MS analysis have subsequently been identified using conventional techniques such as western blotting and immunofluorescence co-localization.

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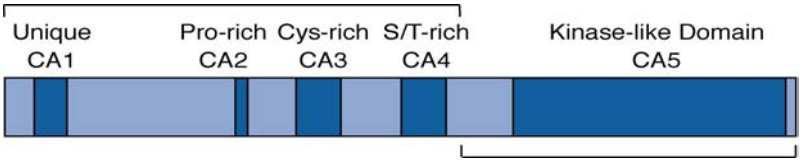
Identification of KSR-interacting Proteins

Programmed cell death 8
MAPK
C-TAK1
Yes-associated WW domain binding protein 2 (WBP2)
14-3-3 ε ζ γ θ ι
Thioredoxin



Associates with both Domains:
Hsp 70
Vimentin

Hsp90
Chaperonin
STI1
p50/cdc27
Hsp40
Hsp27

MEK1, 2
EF1α

Figure 1. Proteins identified that associate with specific KSR domains by a targeted proteomic analysis of immunoprecipitates of full length and truncated forms of KSR.

Reference

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