Requirement for Kinase Suppressor of Ras (KSR) in Ras-mediated Tumorigenesis

Our and other laboratories have defined a new class of upstream signals necessary for normal development and/or oncogenesis via Ras. One component, Kinase Suppressor of Ras1 (KSR1), was originally identified in *Drosophila melanogaster and Caenorhabditis elegans to function* as a positive modulator of Ras-Mitogen-activated Protein Kinase (MAPK) signaling either upstream of or parallel to Raf. The isolation of murine and human KSR1 orthologs with a high level of sequence identity showed that KSR1 signaling is evolutionarily conserved. Attempts to characterize the biochemical and cell biological properties of mammalian KSR1, however, have yielded a confusing and often contradictory picture of the role of this protein in signal transduction. While some studies demonstrated a requirement of KSR1 kinase activity for its action, others indicated the kinase function of KSR1 is dispensable and suggested KSR1 acts primarily as a scaffold protein.

We recently established a 2-stage *in vitro* assay for KSR1 kinase activity in which KSR1 never comes in contact with any recombinant kinase other than c-Raf-1, and defined EGF as a potent activator of KSR1 kinase activity. We showed that phosphorylation of c-Raf-1 on Thr269 by KSR1 is necessary for optimal activation in response to EGF stimulation. KSR1 can also be activated by ceramide, perhaps through the CA3 motif, which is nearly identical to the C1B lipid binding domain of protein kinase C. Ongoing studies are addressing the mechanisms of KSR1 activation through receptor tyrosine kinases and ceramide at the molecular level.

Our laboratory has also generated a mouse knockout of KSR1. Mice deficient for KSR1 develop normally but manifest a defect in Ras-mediated tumorigenesis. Further, inactivation of KSR1 by molecular biologic or antisense targeting causes regression of Kirsten Ras-mediated human pancreatic xenografts in nude mice. Based on these data we have developed a potential therapeutic approach to the treatment of Ras-mediated malignancies, involving selective inactivation of KSR1 by antisense oligonucleotides, and are presently performing pre-clinical toxicologic and pharmacologic studies with the goal of performing a Phase I trial for pancreatic cancer in the near future.

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