

Interrogating Ras Function with Protein Mimetics

Freed, Rebecca

Ras proteins are a family of small GTPases activated by numerous upstream signaling molecules. Mutations in RAS genes (KRAS, NRAS, HRAS) can cause the corresponding Ras protein to be locked in the GTP-bound active state. Ras mutations drive progression of ~30% of all human tumors. However, despite intensive efforts, no therapies for Ras-driven tumors are available and molecular mechanisms of Ras signaling are not completely understood. Elucidating the molecular mechanisms regulating the function of this important oncoprotein is critical for the development of effective therapeutics against a number of currently untreatable Ras-dependent cancers. The goal of this project is to develop rationally designed Ras mimetics to serve as probes of Ras molecular interactions and possible drug leads. Computational analysis revealed three potential interfaces involved in K-Ras dimerization, which is believed essential for signaling. These are composed of helix 3 + helix 4, helix 4 + helix 5, and beta strand 2. I designed and synthesized peptide analogs of these regions and tested their interaction with Ras and downstream effectors through computational analysis as well with microscale thermophoresis, cell toxicity, and proximity ligation assays. Data presented here suggest that helix 5 and helix 3 analogs interact strongly with the K-Ras catalytic domain and helix 5 associates with Ras's poorly characterized effector, AF-6. Additionally, Helix 5 and beta 2 cell-permeable analogs are shown to inhibit the growth of cancer cells. These results support the conclusion that helix 5 is part of the K-Ras binding interface and underscores the utility of synthetic subdomain Ras analogs as powerful tools in understanding the molecular mechanisms of Ras function.

Awards Won:

Fourth Award of \$500