

Protein-based Degradation Strategies against Oncogenic RAS

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Targeted therapies have emerged as a promising cancer treatment strategy by inhibiting proteins specific to cancer cells while preserving healthy cells. RAS proteins play a crucial role in cancer development and are associated with increased tumor growth and invasion. Mutations in RAS genes, especially KRAS G12D, are present in a large proportion of human cancers. However, these proteins have been deemed "undruggable" due to the absence of binding pockets for small-molecule drugs, and the significant challenge of delivering protein-based inhibitors across the cell membrane to reach intracellular RAS.

This thesis focuses on the development of novel protein-based degradation strategies against KRAS G12D, specifically. We first developed a generalizable solubilization strategy to address the low aqueous solubility of proteins that have undergone bioreversible esterification, a permeation strategy that involves raising the cationicity and hydrophobicity of the protein. We then engineered a cell-permeable KRAS-G12D-targeting degrader that consists of an esterified protein-based KRAS-G12D binder, R11.1.6, conjugated to a small molecule ligand of the VHL E3 ligase, VL1. We confirmed the cytosolic entry of esterified R11.1.6-VL1 and demonstrated efficacy in human cancer cell lines through *in vitro* studies. Although modest efficacy in RAS degradation and growth inhibition was observed, this strategy presents a novel paradigm for targeting previously undruggable proteins.

Finally, we build on previous work on intracellularly expressed KRAS-G12D-targeting biodegraders. Unlike the cell-permeable degrader described above, which consists of a small-molecule component, biodegraders are fully protein-based constructs consisting of R11.1.6 conjugated to an E3 ligase itself. They can thus be genetically expressed in cells, eliminating the need for transmembrane delivery. The development of degraders has largely been limited to a trial-and-error approach, with little understanding of the effects of specific design components like linker length, linker rigidity, and target affinity. We utilized high-throughput fluorescence-based screening and regression modeling to determine the relative importance and effect of such design components on RAS degradation, offering several rational design principles that will inform the future development of RAS-targeting biodegraders.

Overall, these findings offer a valuable contribution to the ongoing efforts in developing targeted therapies against RAS and potentially enabling RAS to become a more druggable target.