SEMI-SYNTHETIC K-RAS4B PROTEIN : A PLATFORM FOR INVESTIGATING THE BIOLOGICAL BEHAVIOR OF RAS PROTEIN

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Background: As a lipid post-translational modification on proteins, prenylation is a critical process in regulating protein-membrane interactions. Ras proteins, a class of small GTPases are prenylated. Targeting Ras prenylation is a potential strategy to study Ras-related cancer. FTIs can successfully inhibit cancers driven by H-Ras mutations. However, tumors which primarily contain K-Ras mutations, such as pancreatic adenocarcinoma, have a limited response to FTIs. Therefore, new approaches to understand the mechanism of Ras prenylation in cancer cells is crucial.

Aims: The aim of this project is to construct a semi-synthetic K-Ras 4B protein using a well-known bio-orthogonal protein engineering method called Expressed Protein Ligation (EPL) to investigate the biological behavior of Ras protein, a critical protein involved in cancer. In order to construct a semi-synthetic K-Ras 4B protein using EPL, truncated K-Ras 4B protein (K-Ras 4B $^{1\text{-}174}$-Intein CBD) has been expressed from a transformed BL21(DE3) E. coli. The K-Ras 4B$^{1\text{-}174}$-MESNA thioester has been successfully purified from a chitin affinity column. Solid phase peptide synthesis was used to prepare several forms of the C-terminal hypervariable region(HVR) for different applications. Ligation of those peptides with the aforementioned truncated K-Ras 4B protein thioester yielded several different forms of K-Ras 4B. This aim will be accomplished through the following two projects: A) Developing a method to study cell membrane localization of K-Ras 4B protein in cancer cells, and, B) Construction and structure elucidation of the Ras dimer and analysis of the effect of Ras clustering in cells.