Modulating the eIF4E-4E-BP Protein-Protein Interaction

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Dysregulation of the mechanistic target of rapamycin complex 1 (mTORC1) pathway has been linked to several human diseases including cancer, obesity, insulin resistance, and autism. While attempts have been made to target mTORC1 directly, these therapies have been mostly unsuccessful due to resistance mechanisms which result in dysregulation of cap-dependent translation and cause an overall increase in the translation of oncogenic mRNAs. eIF4E is a downstream effector of mTORC1 and the rate-limiting factor in cap-dependent translation. eIF4E is elevated in approximately 30% of cancers and its link to cancer has been confirmed through knockdown and knockout studies. Cellular eIF4E activity is regulated by the 4E-BPs, which act as gatekeepers of eIF4E by binding and sequestering the protein to prevent cap-dependent translation. The eIF4E-4E-BP protein-protein interaction (PPI) therefore presents itself as a valuable target in treating cancer in addition to other mTORC1-related diseases.

Peptides designed around the sequence of 4E-BP are able to bind to eIF4E, inhibit the growth of cancer cells, and in some cases induce cell death. However, these peptides suffer from poor cell permeability and in every case require conjugation to cell-penetrating peptides (CPPs). "Stapling" of peptides using a hydrocarbon linker is known to increase the drug-like properties of the peptide by stabilizing it to protease degradation and increasing its ability to penetrate cells. Stapling of the eIF4G peptide increases its binding affinity *in vitro*, but no stapled peptides have been published in relationship to this system with any cellular data. Therefore, the aim of this work is to create hydrocarbon stapled peptides designed around the 4E-BP1 peptide sequence and use cell-based assays to determine their ability to prevent the formation of the eIF4F complex and decrease cell growth.

The staple was designed to stabilize a single-turn alpha-helix present in the structure of eIF4E bound 4E-BP1 and maintain an overall positive charge. Conformational restriction of the staple, amino acid sequence, and staple position were investigated to optimize cell penetration and the peptide-eIF4E interaction. All compounds were assessed for effectiveness in triple-negative breast cancer cells. Cell death was judged using a Dapi stain and colony formation assays were used to determine their effects on cell growth. A cap-pulldown assay, in which eIF4E is bound to a resin and its binding partners identified by Western blot, was used to determine the ability of the peptides to directly block formation of the eIF4E-eIF4G and eIF4E-4E-BP1 PPIs. Western blots were also used to identify changes in the phosphorylation of native 4E-BP1 and changes in the expression of c-myc, which is regulated by cap-dependent translation.

Two lead compounds, E->K stapled 4E-BP1 and α-methyl E->K stapled 4E-BP1, were able to penetrate the cell, inhibit colony formation, and inhibit the eIF4E-eIF4G and to a lesser extent the eIF4E-4E-BP1 PPIs. Both compounds decreased cell expression of c-myc and E->K stapled 4E-BP1 inhibited native 4E-BP1 phosphorylation. However, these peptides demonstrated some day-to-day variability in activity which did not appear to relate to compound degradation. They were analyzed using dynamic light scattering and were found to exhibit a tendency to form large aggregates. We hypothesized that preventing compound aggregation would decrease the variability in activity and potentially increase their efficacy in cell assays. Captisol, a cyclodextrin used in some FDA approved drugs, significantly reduced the peptides' tendency to aggregate, but its effectiveness in cell-based assays has not yet been tested.

While 4E-BP1 stapled peptides demonstrate promise in cell-based assays, they require further formulation to be effective. Additionally, further modifications to the peptide structure will be used to increase solubility, cell penetration, and eIF4E binding affinity.