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Beyond the Low Hanging Fruit: Rationally Designed Peptide as Regulators of Protein-Protein Interactions and Their Applications to Human Disease

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Introduction

Protein-protein interactions (PPIs) play a fundamental role in all life events and cellular activities and represent a significant portion of functionally relevant biological interactions; therefore, they are well-established as a class of promising drug targets for their implications in a wide range of biological processes. Peptides and peptidomimetics (modified peptides) can serve as effective PPI inhibitors. Herein we describe the development of novel peptide-based PPI inhibitors for various therapeutic applications [1,2].

Results and Discussion

Based on rational approach we developed peptide PPI inhibitors. We previously found that peptides corresponding to specific regions in proteins, such as (i) docking sites, sites for PPI between a substrate and kinase that is outside of the active/catalytic site of the enzyme, or (ii) regions of homology of otherwise unrelated but interacting proteins, are effective inhibitors of PPIs. Initially using advanced bioinformatic algorithm we identified these regions in target proteins, next, we confirm that the amino acids in these regions are conserved in various species and unique to these proteins. Finally, based on these regions we developed peptides and evaluated their bioactivity in various biological assays [3,4].

Initially we used this approach to target protein kinases, a large and diverse multigene family that catalyze phosphorylation of proteins. Phosphorylation is the most widespread type of post-translational modification (PTM) used in signal transduction, and it is estimated that one-third of the total proteins in a cell may be phosphorylated on at least one residue at any one time. Phosphorylation also plays major roles in numerous cellular functions, including metabolism, proliferation, and survival. Importantly, protein kinases are the second most targeted group of drug targets, and the pharmaceutical industry has dedicated approximately one-third of new drug development programs over the last decade to the development of protein kinase modulators [5,6].

We hypothesized that in the inactive kinase conformation the substrate-specific docking site on the kinase may be masked by an intramolecular interaction, yet upon its activation a conformational change will reveal this docking site, which may be the kinase-binding site on the substrate, and therefore will have similar sequence. Based on this rational we developed novel peptide inhibitors of PPIs derived from the sequence homology of protein kinase C (PKC) and various substrates. Next we synthesized the peptides and evaluated their bioactivity *in vitro*, in cells and in various animal models, demonstrating their efficacy [7]. For example, based on a distal docking site on PKCδ and its substrate, pyruvate dehydrogenase kinase (PDK), a selective inhibitor of PDK docking to PKCδ was developed. The peptide demonstrated high binding to the target protein *in vitro* (K_D ~50 nM), and reduced cardiac injury induced by ischemic events in *ex vivo* and *in vivo* animal models (IC_{50} ~5 nM) [8]. Based on the same rational, peptides that target the PPI sites of PKCδ and other substrates were developed, including glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [9], myristoylated alanine-rich C-kinase substrate (MARCKS), dynamin-related protein 1 (Drp1), insulin receptor substrate 1 (IRS1) [10], and Troponin I [11]. All these peptides demonstrated high specificity and bioactivity. The same exact approach was also used to protein kinase out of the PKC family, such as protein kinase Cdc37 and its binding partner the molecular chaperone Hsp90 [12].

Next, based on the idea that regions of homology of otherwise unrelated but interacting proteins are effective inhibitors of PPIs, we identified regions of homology between proteins that regulate mitochondrial homeostasis. Mitochondria are membrane-bound cell organelles that generate most of the chemical energy needed to power the cell's biochemical reactions. The major mechanisms by which mitochondria maintain their homeostasis are mitochondrial quality control mechanisms such as mitophagy and mitochondrial dynamics including both fission and fusion. Mitophagy, the degradation

and removal of selectively damaged or dysfunctional mitochondria via autophagy, is mediated mainly by the Parkin/PTEN-induced putative kinase 1 (Pink1) pathway. Fission and fusion are mediated by large guanosine triphosphatases (GTPases). Fission, controlled mainly by Dynamin related protein 1 (Drp1) and Fission 1 (Fis1), increases mitochondrial number and can separate damaged parts of the organelle from the functional ones for their selective removal. Fusion, however, is mediated by Optic atrophy 1 (Opa1) and Mitofusin 1/2 (Mfn1/2) and prevents mitochondrial damage by mixing the contents of partially damaged (compromised) mitochondria with healthy mitochondria and allow complementation of dysfunction components [13].

For example, we developed a selective peptide inhibitor of excessive mitochondrial fission, P110, which inhibits Drp1 enzyme activity and blocks Drp1/Fis1 PPI *in vitro* and in cultured neurons. Furthermore, P110 was found to be neuroprotective using a model of Parkinson's disease (PD) in culture by inhibiting mitochondrial fragmentation and reactive oxygen species (ROS) production and subsequently improving mitochondrial membrane potential and mitochondrial integrity. In addition, P110 increased neuronal cell viability by reducing apoptosis and autophagic cell death, and reduced neurite loss of primary dopaminergic neurons in PD cell culture model [14]. The same approach was used to target additional mitochondrial homeostasis proteins, such as Mfn1 [15], Mfn2 [16], transient receptor potential vanilloid 1 (TRPV1) [17], Fis1 [18] and Pink1 [19].

While the prominent role of peptides in controlling important physiological events and in influencing many pathological mechanisms is widely recognized, yet many peptides do not enter clinical trials because of inherent challenges, such as enzymatic susceptibility and membrane impermeability. Peptidomimetics are compounds whose essential elements (pharmacophore) mimic a natural peptide, they are peptide analogs able to mimic the structural elements and functionality of natural peptides retaining the capability to interact with the biological target and produce the same biological effect, while simultaneously addressing the associated undesirable pharmacological properties. In our studies we used several types of modifications to develop peptidomimetic compounds with improved pharmacological properties; these include local modifications, such as the incorporation of non-natural amino acids, as well as global modifications, such as, polypeptide chains that contain a circular sequence, or cyclization [20,21]. In addition, we also modified some peptides to optimize their bioactivity (*e.g.*, introduction of post-translational modifications) [22,23], attached various labels (*e.g.*, biotin or fluorescein isothiocyanate (FITC)) [19,24], and improved their stability (*e.g.*, incorporation of non-natural amino acids and/or cyclization) [25,26].

Peptides and peptidomimetics as drugs show unique characteristics and can be very effective, and their rational design have identified peptides that bind with exquisite specificity and affinity to their targets, therefore having relatively few off-target effects. In addition, they are highly bioactive, very specific, demonstrate low toxicity, and in many cases are developed from natural endogenous scaffolds with known biological activity, thereby making them particularly attractive therapeutic agents [27-29]. Over the years, peptides and peptidomimetics have been evolved as promising therapeutic agents in the treatment of different diseases [21,28,30,31] such as parasitic diseases [32-34], cancer [35,36], diabetes [37], and cardiovascular diseases [8,10,38-42]. There is an increased interest in regulation of PPIs to target intracellular signaling events. Herein we present a rational approach to develop effective pharmacological tools to inhibit PPIs. These peptides and peptidomimetics are useful pharmacological tools *in vitro*, in cell culture and in various animal models, and are promising candidates as therapeutics for various human diseases.

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