Tailoring Peptidomimetics for Targeting Protein–Protein Interactions

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Abstract
Protein–protein interactions (PPI) are a hallmark of cellular signaling. Such interactions occur abundantly within the cellular milieu and encompass interactions involved in vital cellular processes. Understanding the various types, mechanisms, and consequences of PPIs with respect to cellular signaling and function is vital for targeted drug therapy. Various types of small-molecule drugs and targeted approaches to drug design have been developed to modulate PPIs. Peptidomimetics offer an exciting class of therapeutics as they can be designed to target specific PPIs by mimicking key recognition motifs found at critical points in the interface of PPIs (e.g., hotspots). In contrast to peptides, peptidomimetics do not possess a natural peptide backbone structure but present essential functional groups in a required three-dimensional pattern complimentary to the protein-binding pocket. This design feature overcomes many limitations of peptide therapeutics including limited stability toward peptidases, poor transport across biologic membranes, and poor target specificity. Equally important is deciphering the structural requirements and amino acid residues critical to PPIs. This review provides an up-to-date perspective of the complexity of cellular signaling and strategies for targeting PPIs in disease states, particularly in cancer, using peptidomimetics, and highlights that the rational design of agents that target PPIs is not only feasible but is of the utmost clinical importance. Mol Cancer Res; 12(7); 967–78. ©2014 AACR.

Introduction
Protein–protein interactions (PPI) play a fundamental role in cellular signaling pathways. Such interactions are necessary for cell maintenance and healthy metabolic function, which together combine to ensure proper functioning of an organism. Metabolic diseases, and in particular cancer, form a complex network of PPIs that change not only at the initiation and temporarily during disease progression but also in the presence of exogenous therapeutic modulators (1). Understanding the mechanisms behind PPIs, how and why various protein subunits interact under specific conditions, and the consequences of these interactions are fundamental to drug development. Thus, it can be beneficial to either inhibit or promote certain PPIs to realign a system toward homeostasis. However PPIs are complex. Research has recently found that downregulation of a key cellular regulator by treatment with specific siRNAs can lead to reprogramming of gene expression pathways elicited by that protein by redirecting associated proteins to new chromatin-binding sites (1). This demonstrates that modulating a PPI may not necessarily result in realignment to homeostasis but may exacerbate the perturbation by activating alternative signaling pathways. Thus, a more subtle approach, which aims to attenuate a PPI, may prove more effective than completely silencing a given function of a protein (2). Various small-molecule drugs including small synthetic organic molecules, peptides, and proteins have been designed to target specific PPIs. Each possesses advantages and disadvantages with respect to efficacy, specificity, bioavailability, and process of synthesis. Gaining specificity and efficacy is improved in our view by designing molecules based on structural knowledge of PPI interfaces. The effective development and testing of these molecules requires a high-throughput approach. One approach, which addresses robustness, avoids siRNA strategies, and is amenable to high-throughput synthesis and evaluation, is peptidomimetics. These are a valuable class of therapeutic agents, which can be rationally designed to block specific PPIs.

Peptidomimetics (also called peptide mimics) are small organic molecules bearing an identifiable resemblance to peptides or peptide segments of proteins. They can be designed by either modification of an existing peptide or introduction of similar molecules that mimic α-amino acids

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such as peptoids and β-peptides. Peptidomimetics overcome the disadvantages of pure peptide-based drugs, which include limited stability toward proteolysis by peptidases, poor transport properties through biologic membranes such as the intestines and cell membranes, low oral bioavailability, rapid excretion, and poor target specificity resulting from the flexible nature of peptides. In contrast, peptidomimetics offer conformationally restricted structures potentially minimizing cross-target interactions, better transport properties through biologic membranes, improved resistance to degradation by peptidases and other enzymes, and resistance to immune responses (3, 4). To achieve these properties, peptides have been chemically modified to include unnatural amino acid substitutions, backbone amide bond modifications, or rigid scaffolds or addition of hydrophobic residues (5–7). Whereas peptidomimetics do not possess a natural peptide backbone structure, they retain the capability to interact with the same target protein of interest by arranging essential functional groups (i.e., pharmacophores) in a required 3-dimensional (3D) pattern complimentary to a binding pocket in a protein. Thus, a peptidomimetic can be rationally designed to achieve desired effects on cellular signaling pathways by targeting specific PPI motifs. This review focuses on current strategies for using peptidomimetics to target PPIs in disease states, with an emphasis on cancer, as well their potential use as next-generation therapeutic agents.

**Targeting PPIs in Biology**

Understanding PPIs is fundamental to deciphering molecular signaling phenotypes of disease (8), as PPIs regulate critical cellular functions such as cell growth, repair, gene transcription, translation, intra- and extracellular signaling (9). PPIs take place when 2 or more proteins bind together to carry out specific cellular functions or to initiate a cascade of events, which facilitates downstream cellular functions within specific tissues of organs.

**Complexity of PPIs in biology**

More than 5,000 PPIs have been demonstrated to occur using full-length human open reading frames (ORF; ref. 10) and a combination of ORF-based clones and cDNA libraries (11). The recent use of a high-throughput immunoprecipitation combined with mass spectrometry revealed more than 300,000 protein interactions involved in gene transcription and signaling (12) indicating the abundance of PPIs.

Fundamentally, all disease states have roots in aberrant cellular signaling, from cancer, cardiovascular disease, diabetes mellitus, inflammatory disorders, and infectious diseases. Cancer arguably represents the most complex metabolic and genetic disease, with each type displaying unique cellular events in its initiation, progression, and metastases. For instance, tumorigenic B cells in human lymphoma are aided and abetted by Bcl-2 and Bcl-xL proteins, which act to block cell apoptosis by inhibiting pro-apoptotic proteins Bax and Bak (13). In prostate cancer, the androgen receptor (AR) signaling is persistent despite absence of ligand, due to its interaction with a myriad of transcription factors and signaling proteins (14, 15). In each case, the mode of PPI may differ significantly and each interaction potentially forms part of a transient, stable, specific, or nonspecific complex (12). For instance, it is possible for the antiapoptotic actions of Bcl-2 and Bcl-xL to be replaced by other proteins, which bind Bak and Bax. Downregulation of BAG1 (Bcl-2-associated Athanogene-1), a putative coregulator of Bcl-2, decreases Bcl-2 protein expression, but the expression of the proapoptotic protein Bax is not affected. Further analysis shows that BAG3 expression is upregulated to compensate for the deregulation of BAG1, thus stabilizing the protumorigenic activity of Bcl-2 (16).

In prostate cancer, AR is the critical driver, even under conditions of androgen ablation. Maintenance of AR activity following androgen ablation can occur due to amplification of AR, deregulation of the expression of AR coregulators, intratumoral production of androgens, gain-of-function AR mutations, and indirect AR activation by growth factors, cytokines (15). Recently, it was also shown that AR can be activated via estrogen receptor-α (ERα) signaling by binding to the PELP1/ERα complex, thus bypassing its requirement for binding of its cognate ligand (17). In addition, the recent identification of AR variants (ARVs), which form heterodimers with the full-length AR (18, 19), in advanced prostate cancer provides a mechanism whereby PPIs contribute to the progression of this lethal disease. Furthermore, the PPIs between AR and critical coregulators, such as SRC-3, may enable control of additional cellular functions (12). For example, SRC-3, a multifunctional transcriptional coregulator involved in cellular growth programs (20–23) including adipogenesis and energy balance (24) as well as control of mRNA translation of pro-inflammatory cytokines (25), also associates with multiple other coregulators including CBP/p300 and nuclear receptors such as AR and ERα (12).

Cistrome (genome-wide chromatin binding) analysis aided by high-throughput chromatin immunoprecipitation (ChIP) sequencing experiments of various transcription factors shows the genomic location of transcription factor–binding sites is altered when expression of specific protein factors (i.e., pioneer factors required for chromatin accessibility) are enhanced or suppressed (1, 26). Intriguingly, when specific PPIs are disrupted, alternative PPIs may compensate and enable activation of the associated signaling pathway (Fig. 1). Understanding these unique events within disease states is fundamental to designing organic molecules for therapeutic targeting. Rather than completely ablating a protein, it may be beneficial to target a critical surface area that acts as a focal point of interaction between specific proteins to modulate its function. Such an approach could prevent inappropriate proteins being recruited to replace critical “disease-promoting” factors present in a PPI complex.

**Druggable pathways in cancer**

Delineation of signaling pathways involved in the development of cancerous phenotypes, including decreased cell apoptosis, rapid cell proliferation, and increased cell survival,
have enabled development of therapeutic agents targeting critical drivers (27–32). Traditional (and relatively nonspecific) "catch-all" therapies have focused on DNA chelating agents (e.g., cisplatin) and radiotherapy aimed at targeting rapidly dividing cells (33–35). However, poor specificity, a severe side effect profile, and often poor outcomes, particularly in late-stage cancers (33, 36), require re-evaluation of these approaches and the identification of more specific targeted therapies. Identification of a specific target or signaling pathway in cancer can be frustrating, as signaling networks can be inconsistently involved, characterized by promiscuous and overlapping pathways or redundant. The complexity in targeting specific proteins is highlighted by the examples of MSL (responsible for histone H4 lysine 16 acetylation) and NSL (responsible for histone H4 lysine 5 and 6 acetylation) protein complexes, both of which contain the transcriptional coregulator MYST1 but with divergent substrate specificities (37). Thus, targeting MSLs may not affect the cellular or cancer phenotype. Other targets for therapeutic modulation include PPIs found in several of the common pathways linked to cancer (Table 1; ref. 38). Consideration should be given to the existence of alternative pathways to counteract the effect of their therapeutic modulation.

Categories of PPIs

There are several types of PPIs, each with unique binding characteristics and functionalities. Each type requires analysis and deconvolution of signaling effects after altering a specific PPI, either by downregulation of the protein or disruption using synthetic organic molecules. Knowledge of these binding mechanisms with respect to their occurrence in specific PPIs will provide better approaches to targeted therapy. PPIs can be classified broadly into 4 different categories:

1. Protein–protein docking interactions, which occur between similar sized proteins or protein domains and are generally more rigid due to steric constraints (e.g., seen in heterodimers where binding strength is relatively than homodimers; ref. 39).
2. Protein receptor–ligand docking, characterized by tighter binding due to binding pockets within the interaction sites, which can render the interaction rigid. However, the interaction can change conformation to allow induced fit of the ligand within the receptor (40).
3. Rigid ligand with a flexible receptor allows for a larger-than-usual ligand to bind the receptor by virtue of the receptors flexibility. Under normal circumstances, energy penalties are incurred if the ligand–receptor interface requires additional binding (e.g., via van der Waals) due to the large size of the ligand. This is avoided by the rigid nature of the ligand allowing for a more energetically favorable binding conformation (41).
4. Flexible ligand with a rigid receptor is characterized by a smaller ligand compared with the receptor docking site, resulting in a less rigid binding conformation. The flexible nature of the ligand allows numerous binding conformations with the receptor (42). Thus, in such a binding interaction, the challenge is to determine the most energetically favorable conformation (43).
Molecular Cancer Research

Utility and Limitations of Peptide-Based Therapy

Peptides are short chains of amino acid monomers linked by peptide (amide) bonds. The peptide bond is a covalent bond made of the backbone carboxylic acid group of one amino acid and the backbone amine group of another. Peptide therapy was initially based on mimicking an endogenous peptide found to be lacking within a disease state, designed to enhance or replace the effect of a natural peptide. A classic example is the human analogue of insulin, administered to patients with insulin-dependent diabetes. Initially purified from bovine and porcine (44), insulin is now routinely manufactured via recombinant methods as pro-insulin (45). However, critical limitations of insulin and exogenous peptide administration in general, including instability in water and low bioavailability (e.g., poor cell permeability and susceptibility to metabolic degradation), are apparent when administered orally (46). For this reason, injectable pro-insulin is a more efficacious approach. Research is focused on developing less invasive delivery routes including inhalation, transdermal, buccal, and intranasal routes (45).

Other forms of peptide therapy include protein-based hormone administration for cancer treatment. A commonly used strategy for prostate cancer treatment is synthetic luteinizing hormone releasing hormone (LHRH) administration to inhibit androgen biosynthesis in the testes. This occurs via negative feedback when overexposure to LHRH causes lowered LHRH receptor expression, leading to decreased luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion from the anterior pituitary gland, ultimately resulting in decreased androgen synthesis (47, 48). Commercially available forms of LHRH antagonists include abarelix and degarelix (49, 50). However, this form of treatment is limited in its clinical outcomes with respect to castrate-resistant prostate cancer treatment (51). An ideal treatment is tissue- and cell-specific targeted therapy, which allows blockade of critical protumor signaling pathways.

Peptides represent an exciting form of next-generation therapeutic agents as they can be deployed in multiple ways for targeted therapy and implemented to treat malignant tumors. These include using peptides as radionuclide carriers (52–54), vaccines (55), cytotoxic drug carriers (56), or directly as antitumor agents (e.g., inhibiting PPIs). Somatostatin analogues are used in treatment of various tumors as a target for overexpressed somatostatin receptor. The peptide is coupled with a DNA chelator and a radioactive element (e.g., 111In, 90Y, or 177Lu) such that when injected, the radiolabeled peptide targets somatostatin receptor overexpressing cells, delivering the radioactive element directly to tumorigenic cells for eradication (52). Similarly, peptides targeted toward specific receptors can be conjugated with cytotoxic agents aimed at cancerous cells. Classical peptide drugs like LHRH analogues have been conjugated with doxorubicin, a chemotherapeutic agent, which selectively targets cells overexpressing LHRH receptors (57, 58). A corollary to peptide carriers are homing peptides, which are independent of tumor type and target specific molecules in either normal or diseased tissues (59, 60) Targeting angiogenesis may include targeting peptides including RGD and v integrin receptors and NGP receptors, which home in on αv, integrin receptors and aminopeptidase N, respectively (48), which are overexpressed in the neovasculature surrounding the tumors (59). A major drawback of peptide-based drugs is bioavailability. Short synthetic peptides, exclusive of unnatural amino acids, render the molecule unstable in vivo. Peptides are susceptible to degradation by enzymes (e.g., trypsin) or

Table 1. Potential targets for peptidomimetic modulation in common tumorigenic pathways

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Target protein(s)</th>
<th>Cancers</th>
<th>Examples of desired signaling effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK/STAT</td>
<td>EGF receptor, STAT</td>
<td>Head, neck, breast, lung</td>
<td>STAT–STAT homodimer disruption. Decreased STAT-mediated gene activation</td>
</tr>
<tr>
<td>Notch</td>
<td>Notch receptors</td>
<td>Breast, melanoma, medulloblastoma</td>
<td>Notch/ERα complex or other Notch cofactor complex disruption. Decreased ERα signaling</td>
</tr>
<tr>
<td>MAPK/ERK</td>
<td>RAS</td>
<td>Colon, pancreatic, others</td>
<td>Ras–Sos complex disruption. Decreased JUN/FOS-mediated gene activation</td>
</tr>
<tr>
<td>PI3K/AKT</td>
<td>AKT/C-RAF</td>
<td>Glioblastomas, lung, melanomas/breast ovarian, thyroid, others</td>
<td>Ras–RAF complex disruption. Decreased JUN/FOS-mediated gene activation</td>
</tr>
<tr>
<td>NF-κB</td>
<td>REL-A, p50</td>
<td>B-cell, Hodgkin, T-cell lymphomas</td>
<td>NF-κB gene activation</td>
</tr>
<tr>
<td>Wnt</td>
<td>Tcf-Lef</td>
<td>Intestinal adenocarcinomas, myeloid leukemia, prostate</td>
<td>β-Catenin/Tcf-Lef complex disruption. Decreased Tcf-Lef–mediated gene activation</td>
</tr>
<tr>
<td>TGFβ</td>
<td>SMAD</td>
<td>Lung, pancreatic ductal adenocarcinoma, colon, prostate</td>
<td>SMAD cofactor complex disruption</td>
</tr>
<tr>
<td>AR</td>
<td>AR coregulators</td>
<td>Prostate, breast</td>
<td>AR–PELP1 complex disruption. Decreased AR-mediated gene activation</td>
</tr>
<tr>
<td>ERα</td>
<td>ER coregulators</td>
<td>Prostate, breast</td>
<td>A1B1–HER complex disruption. Decreased ERα-mediated gene activation</td>
</tr>
</tbody>
</table>
gastric acids in the stomach, peptidases in the blood and organs like the liver and kidney and poor membrane permeability through the intestine and cell membrane, all of which can contribute to poor half-lives of these molecules. Other considerations include immune response to peptides as well as scaling-up costs for viable commercial use (61).

Finally, in the current climate of drug design, a class of compounds that are mostly targeted toward cancer cells are rationally designed peptide mimics, which are developed on the basis of target-specific binding motifs of PPIs. Such an approach has been tested with respect to prostate cancer, leukemia, and other cancers, in which secondary structural motifs (mostly α-helices) are mimicked to block critical PPIs involved in aberrant cell growth. For instance, AR coregulator interactions recently were targeted to inhibit the interaction between PELP1 and AR in prostate cancer (2). The peptidomimetic D2 was able to inhibit androgen-induced proliferation of prostate cancer cells, block AR nuclear translocation, activation of AR target genes and prostate cancer cell growth in vivo. To develop these agents, non-peptidic scaffolds are synthesized and further modified using a rational design approach to achieve desired specificity, potency, efficacy, and stability. The advantages and disadvantages of protein, peptide, and peptidomimetic drug therapy approaches are summarized in Table 2.

Converting Peptides to Peptidomimetics

Identifying PPI surfaces

Identifying interaction motifs between proteins can be a laborious task for rational drug design. Ideally, analysis of crystal structures by X-ray crystallography provides accurate and visual confirmation of amino acid residues involved at the binding interface. However, obtaining crystal structures by X-ray crystallography provides accurate and visual confirmation of amino acid residues involved at the binding interface. Moreover, X-ray structures often do not reveal deep pockets that mark binding. Furthermore, there remain major structural issues in deciphering contactable surfaces between proteins. A notable consideration includes deciphering the most conformationally stable PPI complex given that all biologic interactions are in dynamic equilibrium (70). X-ray structures often do not reveal deep pockets that mark binding. Moreover, a single crystal structure may only reveal one binding conformation amidst multiple that occur at equilibrium (71) or between

Table 2. Targeting PPIs

<table>
<thead>
<tr>
<th>Therapeutic approach</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>Mimic endogenous function</td>
<td>Bulky</td>
</tr>
<tr>
<td>Peptides</td>
<td>Specific to core PPI hot spots (amphiphilic nature)</td>
<td>Unstable in solution (storage and protein folding problems)</td>
</tr>
<tr>
<td>Peptidomimetics</td>
<td>Stable in solution</td>
<td>Limited biologic function (unlikely to be used as template)</td>
</tr>
<tr>
<td></td>
<td>Resistant to proteases</td>
<td>Some require posttranslational mods (e.g., glycosylation)</td>
</tr>
<tr>
<td></td>
<td>High cell permeability</td>
<td>Low cell permeability</td>
</tr>
<tr>
<td></td>
<td>Low immunogenicity</td>
<td>Susceptible to enzymatic degradation (short half-life)</td>
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<tr>
<td></td>
<td></td>
<td>Unstable in solution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High immunogenicity</td>
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<tr>
<td></td>
<td></td>
<td>Potentially cross-reactive to other PPIs bearing similar conformational hotspots</td>
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<tr>
<td></td>
<td></td>
<td>Based on mimicking generic secondary structures</td>
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transient PPIs. Identifying binding pocket hotspots—small areas of bumps and holes that largely determine binding—can become complicated with respect to multimeric binding interactions. Another problem is that interacting surfaces of proteins typically are many times larger than a small molecule and interactions observed in vitro may not be paralleled in vivo. Therefore, identifying “hotspots” that act as important mediators of PPIs is in essence a trial and error process. Nevertheless, key recognition motifs in a PPI may be verified using targeted mutagenesis of suspected interacting domains and tested via several of the aforementioned assays (Fig. 2).

**Peptidomimetic subtypes**
Conversion of peptides to peptidomimetics is one method to use peptide sequences that have potential as therapeutic agents. Peptides can be converted to stable peptide mimics that display comparable effects to their peptide counterparts but exhibit increased rigidity in structure, improving target specificity, stability, and cell membrane permeability. There are 3 major classes of peptidomimetics (types I–III; Fig. 3), each of which is categorized according to specific chemical modifications such as lactam, spiro, bicyclic bridges, or secondary protein structure mimics (e.g., β-turns and α-helices). However, the current discussion will be based on type I and type III mimetics, which are designed to mimic and target PPIs. Type II mimetics do not mimic PPIs but are likely to be receptor antagonists (6) and are structurally characterized as small non-peptide molecules (72).

**Type I.** Type I mimetics (although not technically classified as peptidomimetics) are short-peptide oligomers designed to replicate the local topography of an α-helical motif found in PPIs (73). Type I mimetics are akin to parent peptides as they may suffer from poor stability and cell permeability. However, type I mimetics differ from parent peptides by exhibiting substitutions such as salt bridges and metal chelators as well as covalent cyclization motifs such as lactam and disulfide bridges to stabilize the secondary structural motif (72). As such, type I mimetics can be thought of as conformationally restricted peptides. Modifications which enable the classification of type I as peptidomimetics are the ones made at the amide backbone structure (e.g., amide bond isosteres). Type I mimetics have been used as inhibitors for aspartic proteases (74), cysteine protease (75), renin (76, 77), and the critical p53/MDM2 interaction present in many cancers (78).

Walensky and colleagues have developed a method for synthesizing stapled α-helical cross-links to mimic interaction peptide motifs of BID (BH3; Bcl-2 homology containing member). The strategy relies on inserting nonnatural olefinic amino acids into peptide sequences, followed by
ruthenium-catalyzed metathesis to form an all hydrocarbon "staple" (79). BID is a proapoptotic BH3-only protein that when activated triggers activation of the multidomain proapoptotic proteins Bax and Bak, resulting in apoptosis (79). However, even with modifications (e.g., lactam bridges) that improve stability of type I mimetics, bioavailability and efficacy issues still exist (72).

**Type III.** Type III mimetics are characterized by highly modified structures that may not contain the basic peptide backbone structure but still retain functional groups necessary for key binding site recognition. In addition, they may represent key characteristics of secondary structures (3, 72). The 2 most common protein secondary motifs are α-helices and β-pleated sheets. In rod-like α-helices, the amino acids arrange themselves in a regular helical conformation where-by the carbonyl oxygen of each peptide bond is hydrogen bonded to the hydrogen on the amino group of the f amino acid 4 residues from the original amino acid. An α-helix is characterized by 3.4 amino acid residues per turn, a rise of 0.54 nm and defined by backbone dihedral angles θ and ψ of −60° and −45°, respectively. A unique feature in the α-helix is that the hydrogen bonds run nearly parallel to the axis of the helix. In β-pleated sheets, hydrogen bonds form between the peptide bonds either in different polypeptide chains or in different sections of the same polypeptide chain. The planarity of the peptide bond forces the polypeptide to be pleated with the side chains of amino acids protruding above and below the sheet. The α-helices are frequently found at the interface of PPIs. More than 30% of protein secondary structures are helical in nature (72). Not surprisingly, strategies have been developed to synthesize helical peptides containing key recognition features of helices as inhibitors of specific PPIs. Type III–based α-helix mimetics can be designed using drastic modifications to the peptide backbone and amino acid side chains and consequently are described as non-peptidic–based peptidomimetics. One approach to designing such α-helix mimetics is via structural scaffolds, which are amenable to high-throughput synthesis (80, 81). The first versions of synthetic non-peptidic scaffolds were oligosaccharide structures, which mimicked projections of the α-helical structure found on the transcription factor GCN4. However, limited to no success was seen with these compounds with respect to GCN4-DNA binding inhibition (82).

Other strategies for disrupting PPIs have been used successfully in targeting well-documented tumorigenic pathways including apoptosis and membrane or nuclear signaling pathways. Hamilton and colleagues developed a non-peptide–based antagonist toward the Bcl-xL protein targeting the hydrophobic cleft formed by the BH1- BH3 domains disrupting the Bcl-xL/Bak interaction (83). The mimetic was designed using a terphenyl scaffold, which in a staggered conformation closely mimics the function of an α-helix. The terphenyl scaffold is designed containing alkyl or aryl substituents at the i, i+3 and i+7 position of the α-helix mimic in addition to carboxylic acid substituents at either end to mimic the Bak α-helical structure. Treatment in HEK293 cells showed decreased binding between Bak and Bcl-xL via protein immunoprecipitation assays (84). The same group also developed benzoylurea scaffolds, which when elongated,
mimics an extended α-helical structure (85). However, thorough in vitro and in vivo studies are lacking for these compounds.

An anthracene scaffold (anthraquinone) was used to mimic the 2 faces of the α-helix of Bim-BH3. Iteration from the anthraquinone scaffold yielded an amphiphilic compound ("compound 6") with dual inhibition of the Mlc-1/Bak and Bcl-2/Bax interactions. Compound 6 was able to induce cell apoptosis in numerous cancerous cells but was innocuous in HEK293 cells (86).

Rodriguez and colleagues improved on amphiphilic helical structures with the use of pyrimidine scaffolds to develop mimics of the α-helical structure composed of the nuclear receptor interaction boxes LXXLL sequence found in nuclear receptor coactivators. A pyrimidine-based mimetic was able to mimic the LXXLL motif on p160 coactivator by forming interactions at the i, i+3, i+4 positions in the hydrophobic groove of the LBD of ERα (87). To improve torsional flexibility and allow induced fit, Hamilton and colleagues further developed diphenylamide scaffolds to mimic the i, i+3, i+4, and i+7 residues to cater for additional interactions from residues flanking the hydrophobic face of the helix.

To disrupt the p53/MDM2 interaction, which is a complex PPI that is deeply buried, Robinson and colleagues developed β-hairpin scaffolds. Side chains of 3 important residues, Phe19, Trp23, and Leu26, of the amphiphilic α-helix project into a hydrophobic cleft of MDM2. The design of this β-hairpin uniquely configures the spatial arrangement of the 2 Gt of residues at i and i+2 on one strand, similar to the α-helical side chains at the i and i+4 residues (88). Surface plasmon resonance showed the mimetic blocked p53/MDM2 interaction.

Oligobenzamide scaffolds

Oligobenzamide scaffolds are pre-organized structures with low molecular weights and can be used to develop peptidomimetics that account for key characteristics of a protein helix. The scaffolding template comprises two or three 3-alkoxy-4-aminobenzoic acids and places 2 or 3 alky substituents corresponding to the side chains of amino acid residues located at the i, i+3, i+4, and i+7 positions in a helix. The oligobenzamide scaffold displays higher torsional flexibility due to the absence of extensive hydrogen bond networks as seen in the trispyridylamide structure despite its similar appearance (81). This facilitates superior α-helix mimicry by arranging the substituents of the oligobenzamide structure in a more staggered fashion as appears in a helix (81).

We recently developed a bisbenzamide-based peptidomimetic to block the interaction between AR and its coregulator PELP1 as a therapeutic approach to prostate cancer. The AR–PELP1 interacting structure was determined on the basis of AR X-ray crystal structure. Analysis of PELP1 revealed 10 consensus LXXLL motifs (where L is leucine and X is any amino acid including leucine) in the protein, which like other nuclear receptor box proteins is a hotspot for interaction (89). Because the LXXLL motif adopts a helical structure, the peptidomimetic D2 was designed with the bisbenzamide scaffold based on computational and molecular docking studies. The structure of D2 was designed to accommodate 2 isobutyl groups reproducing the side chains at the i and i+4 positions of the LXXLL motif, creating a hydrophobic surface for interaction with AR (2).

Other approaches

Aside from α-helix mimetics, other approaches can potentially be used to design peptidomimetics. For example, tethering is an approach whereby an interacting hot-spot between proteins is targeted using a cysteine mutation near the interaction site in the target protein. The mutated protein is probed with test fragments containing disulfide bonds. When a fragment meets the target area, it bonds to the cysteine residue via sulfur bonds, which can then be identified using mass spectrometry. Further fragments can subsequently be screened in this way and the fragments "stitched" together to find a potent molecule (71). Hotspots on the IL2 receptor have been identified by this approach.

A potential but somewhat "random" rational approach to peptidomimetic design includes the construction of peptide libraries using (i) synthetic or (ii) biologic methods. These can be used as lead compounds to be converted into peptidomimetics. The synthetic approach involves an iterative or position scanning process (90). This approach uses coupling chemistry to produce variants of specific peptidic conformations such as α-helices (91) or cyclic disulfide peptides (92), which are then characterized by analytical methods such as nuclear magnetic resonance (NMR) and mass spectrometry. Similarly, phage display is a method of screening a library of candidate peptides by displaying them on the surface of bacteriophage. The display is achieved by fusing a peptide-encoding gene with the gene for a capsid structural protein. In this way, billions of peptides may be screened for binding activity against target proteins. Resulting hits can be selected and the genome sequence of the bacteriophage determined to reveal the sequence of the candidate peptide (93) and binding hit peptides potentially further modified into peptidomimetics, using chemical approaches (94), such as the Smac mimetics. Smac is a mitochondrial protein, which inhibits the apoptotic activity of caspases (95). Smac peptide mimetics are synthesized using a sequence of critical residues of Smac fused with carrier peptides or administered in combination with routinely used chemotherapeutic drugs. For example, quadra- or octa- N-terminal peptides of Smac fused with penetratin, a carrier peptide from Drosophila inhibited PPIs with caspase-3, -7, and -9 and promoted apoptosis in MCF-7 and T47D cells (96). Apoptosis was enhanced in combination with paclitaxel and other drugs. Similarly designed N-terminal Smac mimetics have been shown to promote ubiquitination of target proteins and induce cell apoptosis (97).

Similarly, non-peptidic Smac mimetic designed by computer modeling approaches of the target-binding protein complexed with Smac. Simulation revealed 4 residues on the
N-terminus of Smac interacting with BIR3 domain of a target protein. The C-terminal of this tetrapeptide was critical for binding affinity. On the basis of this interaction, a C2-symmetric diyne was designed to mimic C-terminal binding (98) and was shown to induce apoptosis in multiple cancer cell lines (99–101).

**Improving Potency and Maintaining Specificity**

A key issue in peptidomimetic design is how to present peptide pharmacophores in 3D space so that the structures obtained truly mimic the parent peptide, not just in binding affinity but also in vitro and in vivo biologic function (90). Generally, specific modifications (e.g., substitutions) of the peptide backbone can improve overall binding specificity and potency. These include N-alkylation (facilitating cis-trans amide bond isomerism), Cα-alkylation (stabilizing helical or extended structure), D-amino acid/proline substitution (favoring formation of β-turn structures), peptide bond isosteres (improving metabolic stability), amino acids with cyclic side chains (biasing toward β- or γ-turns), dehydro amino acids and β-alkylation (constraining conformations). Using such modifications in combination to advance the field of peptidomimetic design are type III peptidomimetics, which in principal are based on template scaffolds designed to mimic secondary structures such as α-helices. Such non-peptidic–based mimetics are designed to improve resistance to proteolytic degradation and increase bioavailability. Complex scaffolds can be designed to cater for multiple binding surfaces required to allow binding interactions on more than one side of the mimetic, thereby more accurately targeting a PPI hotspot.

An alternative to inhibiting a PPI via residue contact at an interface is to allosterically regulate a PPI at a position offset from the interface without competing with the protein binding partner to disrupt the PPI (9). This type of mechanism is successfully used by enzymes to regulate their function, that is, a ligand binds at one site (the allosteric site) and induces a conformational change at a distant location, which can cause a change in overall shape and/or conformation at the active site. This type of control can offer greater specificity (64) and its efficacy was highlighted recently with drugs that have potent effects in blocking the interaction between Runx1:CBFβ found in acute myeloid lymphoma (102).

**Limitations of Peptidomimetics**

The primary limitation of peptidomimetic strategies is the potential for a large number of PPIs to be disrupted simultaneously. This may be the case in proteins involving a family of structurally similar domains such as that in steroid hormone receptors, where significant homology exists (e.g., AR, ER, PR, GR, and MR; ref. 103). Interestingly, the ER promoter/enhancer recognition sequence, found upstream of ER-regulated genes, does not share a consensus sequence with AR, PR, GR, and MR promoter/enhancer recognition sequences, like that found in the "universal" MMTV-LTR sequence (104). However, the ER recruits cofactors that are in common with AR such as SRC1 (105), which may have implications for specificity using a template-based peptidomimetic design. A bisbenzamide peptidomimetic that targets a motif may enable the wider targeting of PPIs through that motif but may increase the likelihood of toxicity due to off-target effects. This bisbenzamide may have significant effects on cancer cell proliferation and be used to more broadly target the interactions using a specific motif. Alternatively, a more selective peptidomimetic may be tailored to selectively target a single PPI. Such a peptidomimetic is unlikely to have any toxicity but may not have a significant effect on the cancer cell proliferation due to redundant signaling pathways. Thus, careful refining of the peptidomimetics targeting each PPI is needed to achieve the correct balance between therapeutic efficacy, on-target specificity, and off-target toxicity.

**Conclusions**

Understanding cellular signaling pathways is fundamental in deciphering key PPI in normal and diseased states phenotypes. The rational design of peptide mimics likely will improve with better understanding of PPIs in cellular signaling pathways coupled with improvements in identifying key interacting motifs. These improvements may be in X-ray crystallography techniques or protein sequence mutagenesis methods. The challenge is to design specific PPI modulators that can not only target key protein interactions involved in diseases but also target only the cells displaying aberrant signaling. High-throughput approaches coupled with template-based design offer an efficient strategy for the development of peptidomimetics but target specificity remains a challenge.

**Disclosure of Potential Conflicts of Interest**

G.V. Raj and J.-M. Ahn have patents on peptidomimetics that have been licensed to a pharmaceutical company and have received licensing fees/laboratory support. No potential conflicts of interest were disclosed by the other authors.

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