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Peptide therapeutics: Targeting the undruggable space

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ABSTRACT

Rapid advancements in genomics have brought a better understanding of molecular mechanisms for various pathologies and identified a number of highly attractive target classes. Some of these targets include intracellular protein–protein interactions (PPIs), which control many essential biological pathways. Their surfaces are part of a diverse and unexplored biological space, where traditional small molecule scaffolds are not always successful. While large biologics can effectively modulate PPIs in the extracellular region, their limitation in crossing the cellular membrane leaves intracellular protein targets outside of their reach. There is a growing need in the pharmaceutical field to push the boundaries of traditional drug design and discover innovative molecules that are able to modulate key biological pathways by inhibiting intracellular PPIs. Peptides are one of the most promising classes of molecules that could deliver such therapeutics in the near future. In this review, we describe technological advancements and emerging chemical approaches for stabilizing active peptide conformations, including stapling, hydrogen bond surrogates, beta-hairpin mimetics, grafting on stable scaffolds, and macrocyclization. These design strategies carry the promise of opening the doors for peptide therapeutics to reach the currently “undruggable” space.

1. Introduction

Many essential cellular pathways that are implicated in human diseases are controlled by intracellular protein–protein interactions (PPIs) [1]. Such PPIs could be potential drug targets, and thus the ability of molecules to inhibit specific PPIs has remarkable therapeutic value. Small molecule PPI modulators have already reached clinical studies [2], and with many projects in the discovery phase more will do so in the future. Biologics can successfully target PPIs that are accessible outside of the cell [3] and have become the therapeutic of choice for a number of diseases. Nucleic acid therapeutics (e.g. antisense oligonucleotides and RNA interference products) are one of the most important classes of next generation drugs [4], particularly if current delivery limitations can be overcome. Here, I will focus on recent developments that highlight the potential of peptides for intracellular PPI targeting.

1.1. Why peptides?

The historical definition of druggable targets has been continually evolving. It originally referred to any therapeutic protein target that could be modulated with small, orally available molecules [5]. Small organic molecules are well suited for oral administration because of their stability in the digestive tract and their absorption characteristics - they can enter the circulatory system by passively diffusing across the epithelial cells that line the stomach and intestines, a property that also gives them the ability to enter cells and modulate the functions of specific targets within the cell itself.

For decades, the pharmaceutical industry has been dominated by small molecule drugs. In 1997, Lipinski et al. analyzed the existing database of successful small molecule drug candidates in the clinic and developed a guideline, known as the ‘rule of 5’, to predict and reduce the risk of inadequate oral absorption due to poor solubility or poor permeability [6], by favoring molecules with fewer than 5 hydrogen bond donors, fewer than 10 hydrogen bond acceptors, molecular weight less than 500 Da, and an octanol–water partition coefficient logP no greater than 5. His analysis was

Abbreviations: PPI, protein–protein interaction; HTS, high-throughput screening; PK, pharmacokinetic; mAb, monoclonal antibody; RCM, ring-closing metathesis; aPP, avian pancreatic polypeptide; CCPP, cyclic cell-penetrating peptide; HBD, hydrogen bond donors; CCK, cyclic cysteine knot; HBS, hydrogen-bond surrogate; NRPSs, non-ribosomal peptide synthetases; PEM, protein epitope mimetic; CSA, cyclosporin A; MATCH, macrocyclic template chemistry; DPC, DNA-programmed chemistry; PURE, protein synthesis using recombinant elements; uPA, urokinase-type plasminogen activator; FIT, flexible In vitro translation; RaPID, random nonstandard peptide integrated display; RNAi, RNA interference.

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done partly in response to the development and implementation of combinatorial synthesis and high-throughput screening (HTS), which sometimes led to a bias selection of molecules with increased lipophilicity that were prone to displaying poor pharmacokinetic (PK) parameters. Antibiotics, antifungals, vitamins, and cardiac glycosides, which are often natural products [7], fell outside the 'rule of 5'. Various empirical criteria [8] along with the 'rule of 5' have been applied to the small molecule drug space for more than a decade and have successfully reduced the prevalence of drug candidate attrition due to poor PK. Rational design coupled with novel screening technologies has resulted in small molecule hits for traditional, tractable extra- and intracellular targets such as receptors, ion channels, and enzymes. Yet, the number of new small molecule entities approved per year has remained flat [9,10].

Despite their unique and attractive ability to penetrate cells via passive diffusion, it is a challenge for small of molecules to address novel targets such as intracellular protein–protein interactions [11]. The inherent properties and diversity of PPI interfaces make it extremely difficult to design small molecule inhibitors, even though in recent years we have witnessed a number of successful examples of small synthetic molecules modulating PPI [2,12–16].

The most obvious and unique feature of PPI is the size of the interaction surface. Typical PPI interfaces span the contact area of 1500–3000 A^2, while the interfaces for protein-small molecules are only in the range of 300–1000 A^2 [17]. In addition, most PPI interfaces are relatively featureless, lacking pre-formed and well-defined hydrophobic cavities which can fully accommodate a small molecule ligand. Such single, deep binding pockets that entirely surround the bound ligands are usually found on traditional 'druggable' targets, and on average, they occupy a volume of ~270 A^3 [18] (Fig. 1). In contrast, a PPI interaction surface is a collection of a few smaller binding pockets scattered across the interaction interface, each with a volume of about 100 A^3 [19]. It has been shown that only some of these interactions, called 'hot spots' [20], are essential for affinity as they contribute the majority of the total interaction energy. In order to achieve good affinity and competitive binding, a PPI inhibitor should be large enough to simultaneously interact with multiple 'hot spot' patches and gain a significant part of the distributed free energy [21]. In some cases, when 'hot spots' are localized in close proximity to each other, or when proteins have adaptive binding surfaces [16], they can be effectively modulated by small molecule scaffolds [22,23]. However, in general, molecules with larger contact surfaces such as biologics and peptides are necessary to achieve nanomolar potency at the PPI interface. Not surprisingly, structural analyses of small molecules that have successfully inhibited PPI show that they differ from known small molecule drugs: they tend to have higher molecular weight and more complex topology than typical 'drug-like' molecules adhering to the 'rule of 5' [17].

Another challenge presented by PPI targets is the need for new and diverse scaffold libraries to allow for better sampling of the PPI chemical space. In the absence of small natural substrates or ligands, high-throughput screening (HTS) methods are often used to discover small molecule inhibitors. However, screening is rarely successful. One of the reasons is lack of diversity in the commercial small molecule compound libraries used for screening. The chemical space of the existing libraries is strongly influenced by binding pockets of traditional targets (e.g. enzymes, G-protein-coupled receptors). Consequently, 'drug-like' compound libraries are not diverse enough to contain PPI surface-compatible molecular scaffolds. A number of studies have analyzed known PPI inhibitor molecules and delineated some of the features that differentiate PPI inhibitors from 'drug-like' molecules: more hydrophobic, rigid aromatic scaffolds combined with charged or polar groups, larger in size, macrocyclic, higher number of chiral centers, and three-dimensional in bound conformation [17,18,24–26]. A better understanding of the chemical space of PPI inhibitors will help to create new, focused chemical libraries that could ensure more successful HTS screening in the future [27].

It is evident that novel targets such as intracellular PPIs pose challenges that cannot be answered simply by small, orally available molecules. Large and diverse PPI surfaces, which feature complex topologies of multiple low energy interaction sites, are well-complemented and effectively modulated by protein-based biologic drugs. Due to their greater size and well-defined three dimensional conformations, biologics can bind their protein targets with high affinity and remarkable selectivity. Today, several monoclonal antibodies (mAb) that target extracellular proteins are among the blockbuster therapeutics on the market [28]. Modern developments in drug delivery technologies have allowed non-oral delivery systems, such as injectables, to become acceptable alternative routes of drug administration. Protein therapeutics have expanded the historical definition of "druggable" targets. However, their large size restricts their diffusion across the cell membranes, hindering their ability to reach intracellular targets.

Peptides, which are distinguished from proteins based on their smaller size (50 amino acids or less), mediate various essential biological functions, such as signal transduction, heart rate regulation, food intake, and growth. Natural peptides such as insulin, oxytocin, and cyclosporine are successful drugs [29]. Similar to biologics, peptides can bind large protein targets with high potency and great selectivity, which translates into fewer off-target side effects and less potential for toxicity than small molecule drugs [30]. Unlike small molecules which often trigger side effects by producing toxic metabolites that accumulate in different organs [31], peptides degrade into amino acids, which minimizes the risk

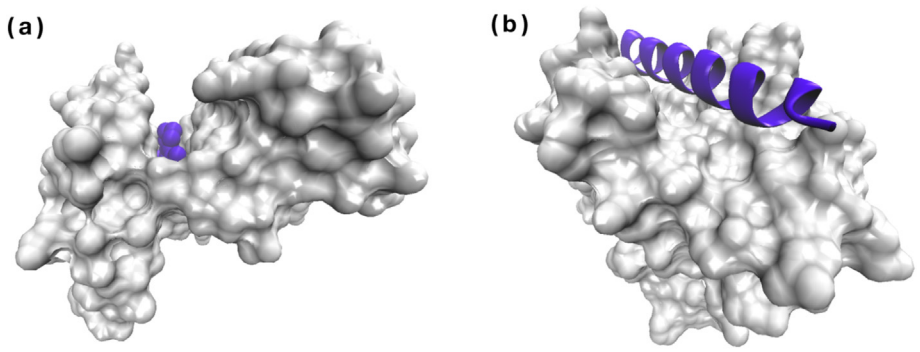

Fig. 1. Examples of two different binding modes: (a) Small molecule inhibitor bound to the EPHA4 receptor tyrosine kinase displays compact binding (PDB: 2XYU), (b) the PPI interface between Bcl-2/BID domains (PDB: 2VOI) is an example of extended binding.

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