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Evolution and Exploration of Drug Development Using Therapeutic Peptides for Covid - 19

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ABSTRACT: The whole world has immersed in a pandemic. It is caused by the emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Though no drug (exclusively for this disease) has been approved, several vaccines are recently available. In this paper, our focus is on the main virus-based and host-based targets to discover new drugs for this devastating disease. All CoV enzymes and proteins involved in viral replication and the control of host cellular machinery are potentially druggable targets in the search for therapeutic options for SARS-CoV-2.

KEYWORDS: Peptides, Protein, Amino acid, Hydrophobic, Therapeutic, Covid-19, SARS CoV-2, Drug Discovery

I. INTRODUCTION

The pandemic COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This is a highly pathogenic human coronavirus (CoV). It was first reported in Wuhan, China, where pneumonia of unknown cause was detected in December 2019. This novel CoV belongs to the Coronaviridae family, along with SARS-CoV and the Middle East respiratory syndrome coronavirus (MERS-CoV). The three of them are zoonotic viruses and have in common their ability to cause severe infection in humans, in contrast to other human CoVs (HCoV-NL63, HCoV-229E, HCoV-OC43, and HCoVHKU1), which are responsible for mild respiratory tract infections.

Protein-protein interactions (PPIs) are the foundation of every cellular process. These biochemical processes have activated receptors. They indirectly or directly regulate a series of enzymatic activities. Drugs that bind specifically to such receptors can act as agonists or antagonists, with downstream consequences on cellular behavior. Peptides interfere with PPIs and are in high demand in pharmaceutical industries. Peptides have the potential to modulate disease-associated protein interactions. Proper identification of targetable disease-associated PPIs and optimization of peptide drug binding characteristics will play a very important role.

II. RELATED WORK

In [5] Using a chromatographic technique (HPLC) and ideal experimental conditions, the scientists assessed the protein quality of a high protein raw component (corn gluten). They employed pre-column derivatization, separation by high-performance liquid chromatography (HPLC), and UV detection in the investigation. The next step was to use the improved technique to identify the amino acids from raw, high-protein products that are frequently used in animal feeding (corn gluten, 61 percent CP). Six distinct days were dedicated to the analysis of a set of six samples, each of which was produced in two.

In [6] authors assess data from therapeutic drug monitoring (TDM) which gives the practitioner more understanding of the variables affecting the patient's response to pharmacological therapy. When a patient does not respond to a typical therapeutic dose, for instance, measuring the plasma level can help distinguish between a noncompliant patient and a real non-responder. TDM also offers helpful information on how drug use patterns vary from person to person and how drug use changes as a result of changes in physiological state or disease processes.

In [7] the RPLC, IEC, and SEC HPLC modes that can be used to analyze the required protein molecules are examined by the authors. When creating a method for protein molecule analysis, one should take into account the many factors included in these diverse modes. There are various detectors available for these various HPLC modes. Depending on the goal of the procedure and different protein properties, detectors can be chosen.

In [8] The authors review: (i) a comprehensive overview of peptide drug development and the underlying technologies used to date, and (ii) an up-to-date review of key developments in computational modeling peptide-protein interactions (PepPI) with the goal of helping experimental biologists exploit appropriate splicing methods to advance peptide interference strategies against PPIs. Despite recent advances in protein-protein and peptide-protein structural computational modeling, major challenges remain.

In [9] authors focused on the main viruses and servers goals which can guide efforts in pharmacogenomics discovery. New medicine for this terrible disease of SARS-CoV-2. In principle, all CoV enzymes and proteins involved in viral replication and control Cell hosts are potential drug targets in study treatment options for SARS-CoV-2. Given the urgency of the COVID-19 pandemic, authors feel the repurposing of approved drugs is the only alternative to find a timely effective treatment. Drugs currently under clinical trials were initially approved for other indications. However, the past and present coronavirus outbreaks require preparedness not only for the current situation but also for a future potential re-emergence of novel coronaviruses. In this sense, authors feel it is of utmost importance to design drugs acting as pan-coronavirus antivirals or through a multi-target approach to avoid a lack of effectiveness by viral mutation escape.

In [10] different modes of chromatography that can cover almost every separation case are considered. Additionally, an outlook to a very recent continuous chromatographic process (namely Multicolumn Countercurrent Solvent Gradient Purification, MCSGP) and future perspectives regarding purification strategies are considered.

In [11] authors summarize the efforts and achievements in peptide drug discovery, production, and modification and their current applications. They also discuss the value and challenges associated with future developments in therapeutic peptides. The discovery of potential therapeutic peptides is the first step in peptide drug development, followed by chemical or biological peptide synthesis and sequence modification to improve its pharmacological properties. Here authors summarize the fundamental technologies utilized for peptide production and modification.

III. PROBLEM IN EXISTING PEPTIDES

As natural amino acid-based therapeutics, therapeutic peptides have two intrinsic drawbacks: membrane impermeability and poor in vivo stability, which represent major stumbling blocks for peptide drug development

1. Peptides have weak membrane permeability. The membrane permeability of peptide drugs depends on multiple factors, including peptide length and amino acid composition. Peptides are generally unable to cross the cell membrane to target intracellular targets, thus limiting their applications in drug development. Lau et al. reported in 2018 that >90% of peptides in active clinical development targeted extracellular targets, including G-protein coupled receptors (GPCRs), gonadotropin-releasing hormone (GnRH) receptor, Glucagon-like peptide 1 (GLP-1) receptor

2. Peptides have poor in vivo stability. Natural peptides consist of chains of amino acids joined by amide bonds, but lack the stability conferred by secondary or tertiary structures. The amide bonds can be easily hydrolysed or destroyed by enzymes in vivo, upon exposure to the environment, without any protection. These inherent chemical properties make the peptides chemically and physically unstable, with a short half-life and fast elimination in vivo.

These present both challenges in peptide drug development and also opportunities and directions for peptide drug design and optimization.

Peptide drugs have been applied to a wide range of diseases, such as diabetes mellitus, cardiovascular diseases, gastrointestinal diseases, cancer, infectious diseases, and vaccine development. Considering their huge therapeutic potentials, market prospects, and economic values, we expect therapeutic peptides to continue to attract investment and research efforts and to achieve long-term success.

Our work intends to analyze various possibilities of therapeutic drug discovery using peptides therapeutic properties.

IV. PROPOSED SOLUTION

The generally poor cell membrane permeability of peptides has limited their application against inaccessible intracellular targets. Peptide therapeutic development has largely focused on extracellular targets due to this limitation. Improvement of membrane permeability or development of strategies that facilitate active intracellular uptake will be critical for successful peptide-based targeting of intracellular PPIs. Potential strategies for improving intracellular uptake include modulation of the hydrophobicity and electrostatic charges to improve passive uptake, or conjugation of the active drug

peptide to a cell-penetrating peptide (CPP) to facilitate its active transport.

Bioavailability of peptide biotherapeutics is usually greatly enhanced when they are more watersoluble, as effective serum concentrations can be easily maintained. Optimization of aqueous solubility is however a largely empirical process, with experimental identification of unnecessary hydrophobic amino acids which can be replaced with charged or polar residues to modulate the P_i while maintaining bioactivity.

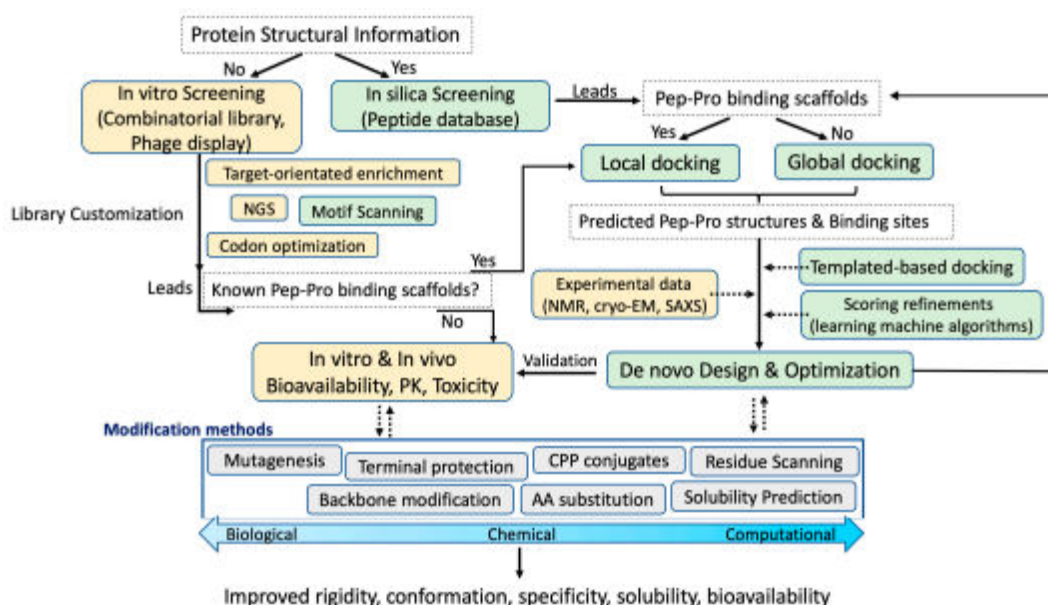


Figure 1 :Modular view of the peptide drug development cycle

This flowchart provides ways proposed for drug discovery. Boxes in green color indicate computational methods; gold are biological methods; grey are commonly modification methods applied for improving peptide bioactivity. The blue two-headed arrow represents the modification methods that are relatively more biological, chemical or computational. White dashed boxes are criteria for accessing which method can be chosen next depending on availability of information. Solid or dashed arrows indicate direct or optional connections between methods, respectively.

The discovery of potential therapeutic peptides is the first step in peptide drug development, followed by chemical or biological peptide synthesis and sequence modification to improve its pharmacological properties. Here we summarize the fundamental technologies utilized for peptide production and modification.

PEGylation of peptides and proteins:

Azide or acetyl groups are introduced into therapeutic peptides and proteins by genetic code expansion to allow downstream PEGylation modifications. Short protein and peptide therapeutics produced by genetic code expansion also have a short half-life because of their poor pharmacokinetics, including fast serum degradation and quick elimination. Attaching a polymer is one approach for extending the half-life of protein therapeutics. PEG is formed by repetitive units of ethylene oxide and is a non-biodegradable, non-toxic, low-immunogenic polymer. PEGylation can increase the effective molecular weight of proteins to reduce their renal clearance by kidney filtration.

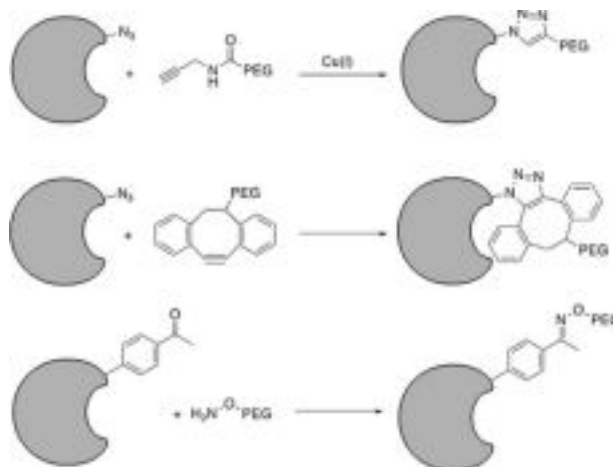


Fig.2 PEGylation of therapeutic peptides and proteins via genetic code expansion.

Overcoming Intrinsic Drawbacks of Peptide Drugs:**Termini Protection :**

Proteolysis can potentially take place from both N- and C-termini of a peptide by up to 500 proteases and peptidases including serum aminopeptidases and carboxypeptidases [16]. It is well-documented that different amino acid residues at N- or C-terminal will result in different degrees of proteolysis and degradation. For example, residues such as Met, Ser, Ala, Thr, Val and Gly at N-terminus are more resistant to degradation in plasma, whereas peptides rich in Pro, Glu, Ser and Thr are more susceptible [17]. If the N- or C-terminal sequences can be modified while maintaining the required targeting specificity and affinity, such modification can reduce proteolytic degradation and improve bioavailability [18]. Similarly, as long as such modifications permit proper functioning of the drug, C-terminal amidation or N-terminal acetylation can also be used to improve in vivo stability [19]. Modification with unnatural amino acid analogs may also serve the same purpose.

Non-Chemical Methods: Identifying Critical Residues

For biologists, the choice of chemical modification often requires expertise in chemistry through collaborations. There are, however, a number of methods that can be approached easily and yet are important for biological study of peptide drug design. First, it is necessary to identify the minimum amino acid residue(s) essential for peptide activity. This can be achieved by repetitive truncating amino acids from either the N- or C-terminus of a lead sequence to ascertain the critical core peptide motif needed for biological activity. Secondly, a classic screening method called alanine scanning can be used to determine the contribution of individual amino acids to biological activity of the peptide [20]. By screening the biological functionality of a library of peptides where individual amino acids have been substituted with alanine, it is possible to identify critical amino acids. Alanine is used for the substitution because its small, uncharged side chain doesn't interfere with the function of adjacent side chains [21].

Synthetic Amino Acid Substitution and Backbone Modification

Although enantiomer amino acid (D-amino acid) substitution has been a common approach to protect peptides from protease degradation, the conformational changes created can compromise the biological activity of the original L-peptide [27]. In addition to the use of D-amino acids, α -methylation and N-methylation have also been widely used. While α -methylation of amino acid provides the advantage of maintaining the side-chain at its original spatial orientation, which is critical for helical peptides; N-methylation has been proven beneficial for enhancing solubility and reducing undesired polymerization [27,28]. Such side-chain functionality-modifying strategies have evolved peptide secondary structures and yielded new peptidic molecules that are referred to as peptidomimetics. Further information on the chemistry and applications of β -D-amino acids, α -N-methylations, or backbone-modified semicarbazide-peptides, peptoids and peptidomimetics can be found in these reviews [29–33].

The protease resistance of peptides can also be improved by peptide cyclization. There are a number of strategies for generating cyclized peptides. These include head-to-tail cyclization, which generates a peptide bond between the original N- and C- termini. The amino group on lysine side-chains can be reacted with aspartic or glutamic acid side chains or the free C-terminus, forming an amide bond. Alternatively cysteine pairs can be reacted to form a disulphide bond between the side chains. These strategies can protect termini and restrict conformational flexibility of peptides,

maintaining them in their bioactive conformation [34]. Cyclization between side-chains, in particular, has been proven effective in optimizing conformational stability for helical peptides. The cyclized peptide drug ATSP-7041 is an example of a recent success [35]. This side-chain cyclized α -helical (stapled) peptide is able to selectively bind to and inhibit MDM2/MDMX, thus activating p53-dependent tumour suppression [6]. Such strategies of targeting PPIs are of great therapeutic potentials as there has been vast amounts of information available on disease-related PPIs in the literature but peptide-based inhibitors are only emerging to mature in the past decade.

Computational Methods for Improving Aqueous Solubility and Membrane Permeability

The generally poor cell membrane permeability of peptides has limited their application against inaccessible intracellular targets. Peptide therapeutic development has largely focused on extracellular targets due to this limitation. Improvement of membrane permeability or development of strategies that facilitate active intracellular uptake will be critical for successful peptide-based targeting of intracellular PPIs. Potential strategies for improving intracellular uptake include modulation of the hydrophobicity and electrostatic charges to improve passive uptake, or conjugation of the active drug peptide to a cell-penetrating peptide (CPP) to facilitate its active transport.

Bioavailability of peptide biotherapeutics is usually greatly enhanced when they are more water soluble, as effective serum concentrations can be easily maintained. Optimization of aqueous solubility is however a largely empirical process, with experimental identification of unnecessary hydrophobic amino acids which can be replaced with charged or polar residues to modulate the pI while maintaining bioactivity [36,37]. Recently, two support-vector machine (SVM) machine-learning bioinformatic tools were developed to expedite this process [38]. ccSOL omics offers a large-scale calculation of solubility for not only a proteome-wide prediction, but also the identification of soluble motifs within any given amino acid sequence [39]. PROSO II is another SVM learning based online tool to predict solubility based on physiochemical properties of the primary sequence such as the degree of hydrophobicity, hydrophilicity and secondary structural propensities in coil, helix or sheet [40].

Membrane Protein-Facilitated Intracellular Peptide Uptake

Cyclic peptides have superior proteolytic resistance and structural stability than linear peptides. Tremendous efforts have been made towards developing of cell-permeable cyclic peptide drugs to block PPIs. Short CPP motifs have been rationally conjugated to otherwise cell-impermeable cyclic peptides to facilitate their intracellular uptake. This delivery strategy has been applied more generally to develop bicyclic peptide drugs comprised of one membrane-crossing CPP moiety and one cyclic peptide PPI inhibitor, while maintaining target specificity and affinity [52]. The oncogenic Ras-Raf interaction was blocked by a bicyclic peptide inhibitor that significantly impeded MEK/ATK signaling and led to apoptosis in lung cancer cells [53]. While CPPs are not themselves immunogenic, bioactive peptide-conjugated CPPs can sometimes induce an immune response, which may limit their application against some targets [54].

V. CONCLUSION

Due to the distinct biochemical characteristics and therapeutic potential, peptides have become a very important therapeutic agent in recent years. Peptides works for small molecules as well as large biologics but they often suffer from membrane impermeability and poor stability in vivo. It is due to the intrinsic limitations of amino acids. To overcome these drawbacks, extensive research for the discovery, production, and optimization of peptide drugs has been carried out.

This work has analyzed and provided a proper pathway to therapeutic drug discovery using computational and other methods available and arranged it in a logical manner which would help further researchers.

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