SOME FACTORS TO CONSIDER IN THE DE NOVO DESIGN OF ANTICANCER PEPTIDES

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Abstract

Background: More than 10 million new cases of cancer are diagnosed each year and the incidences continue to increase, making it one of the top causes of death in the world. Anticancer peptide (ACP) therapeutics is an emerging and promising strategy in the treatment of cancer as it offers several advantages over conventional chemotherapy.

Objective: The objective of this study is to provide a comprehensive overview of the structural properties of ACPs and the associated molecular descriptors to be considered in their de novo design.

Methods: This study reviewed the factors to be considered in the de novo design of ACPs. A literature search in Google Scholar and PubMed was conducted for the relevant original research and review articles on the structural properties of ACPs. Related articles published in English were selected, studied and discussed. Results: As revealed by the selected articles, the key factors to be considered in ACP design include druggability, allergenicity and toxicity, stability, tumor-homing ability, and cell-penetrating ability. Utilizing in silico, in vitro, and in vivo approaches, and these parameters may be assessed, and they considerably increase the ACP's efficacy and safety.

Conclusion: ACPs have shown great promise in the treatment of cancers. With good design, they can emerge as the best alternative to the small molecule drugs used for chemotherapy.

Keywords: Cancer, Anticancer peptides, Design, Descriptors

Introduction

More than 10 million new cases of cancer are diagnosed each year and the incidences continue to increase, making it one of the top causes of death in the world. Several anticancer drugs are available; however, cancer treatment is still not very effective [Gautam et al, 2014; Rowaiye et al, 2020]. There is an urgent need for new anticancer drugs or therapeutic strategies due to the harmful side effects and multidrug resistance of conventional chemotherapy. Due to their distinct mechanism and other remarkable features, peptides have emerged as attractive molecules for new anticancer therapy [Huang et al, 2015]. Peptides are easy and cheap to synthesize, easy to modify, have high tissue penetration and also have a lower propensity to lead to drug resistance [Xie et al, 2020]. When compared to the actual chemotherapeutic drugs available for cancer treatment, peptides are distinct molecules that exhibit a range of mechanisms of action [Gaspar et al, 2013] which include angiogenesis inhibition [Acero, et al, 2017], membrane disruption [Nyström & Malmsten, 2018], and tumour growth inhibition [Habault, & Poyet, 2019].

Since the development of insulin therapy in the 1920s, peptide therapies have made a significant contribution to medical practice. Peptide drugs continue to enter clinical development at a steady rate [Lau & Dunn, 2018]. Interestingly, only a small portion of the vast amount of research that has been conducted on the potential of bioactive peptide as a novel class of drugs has been effectively converted into clinical trials. [Yap & Gan, 2020]. This is because therapeutic peptides have various limitations, such as short half-life, low oral bioavailability, poor membrane permeability, and metabolic instability which decreases their efficacy [Kumar et al, 2020; Craik, et al, 2013]. Peptide therapeutics tend to be exquisitely specific for their targets due to many more interactions with them, whereas conventional small molecule drugs may suffer from reduced target selectivity due to their small size, which frequently manifests in human side-effects [Craik,et al, 2013].

Anti-cancer peptides (ACPs) are a group of low molecular weight, cationic peptides with anti-tumor activities such as inhibiting the proliferation and migration of tumour cells, as well as preventing the development of tumour blood vessels [Xie et al, 2020]. ACPs can be obtained from different sources of nature such as bacteria [Karpiński & Adamczak, 2018], marine organisms [Malaker & Ahmad, 2013], plants [Lath, et al, 2022], and animals [Wang et al, 2017]. Based on their primary structures, ACP can be classified into 4 categories namely: α-helical, β-pleated sheets, random coil, and cyclic [Xie et al, 2020]. In the search for safe and effective peptides against cancer, natural ACPs could be modified or ACPs could be designed de-novo.

This review aims at highlighting the major factors to consider in the de novo design of anticancer peptides for efficacy and safety.

2.0 Materials and Methods

A literature search in Google Scholar and PubMed was conducted for the relevant original research and review articles on the structural properties of ACPs, published within 2006–2022. The search was conducted using key

word combinations such as “Anticancer peptides”, “peptides AND solubility”, peptides AND solubility”. peptides AND proteolytic stability”, peptides AND toxicity”, peptides AND charge”, peptides AND cell penetration”, etc. Articles related to the subject published in English were chosen, studied and discussed. A total of 74 articles published in English were selected.

3.0 The design of Anticancer peptides

In the design of de novo ACPs, the following properties and their associated molecular descriptors should be considered. They include druggability, allergenicity and toxicity, stability, tumor-homing ability, cell penetrating and target-binding ability. Design can be enhanced by the use of in silico tools for prediction and evaluation through in vitro and in vivo methods.

3.1 Druggability: Reduced peptide bioavailability can be caused by poor absorption, distribution, metabolism, and excretion (ADME) properties [Foltz et al, 2010] and these include quick clearance, short half-life, low permeability, and occasionally low solubility [Di, 2015]. Therefore, for safety and efficacy, ACP design should seek to increase druggability by increasing solubility and permeability, reducing proteolysis and renal clearance, and prolonging half-life [Di, 2015]. Presently, the knowledge of the ADME properties of therapeutic peptides is not as advanced as it is for small molecules [Roller, 2017]. Therefore, a more detailed knowledge and evaluation of structure-activity relationship is necessary in peptide design [Naylor et al, 2017].

3.1.1 Solubility: The solubility of peptides is a key factor that affects their bioavailability. Proteins are naturally expressed at amounts close to their solubility limits and hence being weakly soluble, protein aggregation must be actively prevented [Sormanni & Vendruscolo, 2019]. The process of aggregation, which is a type of protein folding, involves intermolecular interactions resulting in the development of massive, insoluble assemblies (fibrillar or amorphous). It is therefore important that peptide design should have the ability to precisely predict the likelihood of the formation of aggregates [Trainor, et al, 2017].

Amino acid composition which determines the overall peptide charge is frequently found to be the parameter that best predicts solubility [Trainor, et al, 2017; Zapadka, et al, 2017]. A rule of thumb in designing soluble peptides is to ensure that at least 20% are charged residues (ThermoFisher, 2022). There are several webservers that use amino acid sequences to predict peptide aggregation using computational algorithms [Santos et al, 2020]. Also, in vivo, aggregation can be detected by tryptophan fluorescence spectroscopy. Positive results show that the formation of peptide aggregates within 48 hours significantly increased the intensity of emission spectra [Lin et al, 2016].

The hydrophobic composition of a peptide also determines its solubility. Peptides with more than 50% hydrophobic residues may not dissolve in aqueous solutions or may only dissolve partially [ThermoFisher]. Peptides have the propensity to aggregate with high hydrophobicity. It has been discovered that mammalian model membranes were destroyed by peptides with four Leu residues, but not by ones with the equivalent Ala residues [Yin, et al, 2012]. The solubility of hydrophobic peptides found in alpha-helical regions of proteins have been increased by swapping out Ala residues for alpha-aminoisobutyric acid (Aib) residues [Narita et al, 1985].

In peptide synthesis, maintaining the solubility of peptides before, during, and after purification, remains a formidable challenge. The pH of the excipient is crucial in enhancing peptide solubility [Zapadka et al, 2017]

3.2 Allergenicity and Toxicity: Only a small portion of the vast amount of research that has been conducted on the potential of bioactive peptide as a novel class of drugs has been effectively converted into clinical trials. Allergenicity is a major factor that could be responsible for this. Numerous peptides have been found to allergenic due to the complex interactions with the host environment [Yap & Gan, 2020]. A preliminary in silico prediction, screening, and analysis of this feature is provided by bioinformatics. The in-silico tool, AlgPred can be used to evaluate the peptide's allergenic susceptibility. With the help of bioinformatic tools, this crucial parameter should be taken into account when evaluating the therapeutic potential of ACP [Yap & Gan, 2020]. Synthesized peptides can be detected in solution by using mass spectrometry for targeted quantitation [Croote & Quake, 2016].

The toxic potential of an ACP can be determined by its hemotoxic and cytotoxic activities [Goyal et al, 2021]. The apparent inherent propensity to cause hemotoxicity is another reason that has prevented therapeutic peptides from further clinical development. Therefore, a crucial step in creating ACP is to take their hemolytic activity into account [Speck-Planche & Kleandrova, 2022]. In this context, computational (molecular dynamics simulation and molecular docking) and machine learning (random forest, support vector machines (SVM), and artificial neural networks) techniques play a crucial role in hastening the discovery or design of non-hemolytic ACP [Speck-Planche & Kleandrova, 2022]. An in-silico tool named HemoPred can be employed to predict the hemolytic activity of the peptides. HemoPred is a sequence-based predictor that uses the composition of amino acids, dipeptides, and other physicochemical descriptors. Hemopred reveal the significance of hydrophobic and cysteine residues on the hemolytic activity of peptides [Win et al, 2017]. Studies reveal that high hydrophobicity of peptides is often connected with substantial hemolytic activity [Huang et al, 2012].

This should be validated by in vitro testing to determine the hemolytic concentration that causes the hemolysis of 50%

of RBC [Speck-Planche & Kleandrova, 2022] or the hemolytic index using common standardized assays [Bacalum, & Radu, 2015]. ACP must show low toxicity against erythrocytes. Their ability to lyse human red blood cells should be tested using melittin as positive control [Oddo & Hansen, 2017].

3.3 Stability: Rapid proteolytic degradation, immune system recognition, receptor-mediated clearance, renal clearance, and hepatic metabolism of peptide drug candidates are the different mechanisms that contributes to the short half-life of peptides and this is a major challenge in the development of peptide therapeutics [Zaman et al, 2019; Li et al, 2018]. Many peptides have short half-lives in vivo, usually just a few minutes to hours, which act as a barrier to their therapeutic use [Tan et al, 2018]. The majority of peptides do not proceed to the duodenum and jejunum's absorption stage because they are frequently subject to substantial hydrolysis in the gastrointestinal tract by brush border, small intestine, and stomach peptidases. [Foltz et al, 2010]. Consequently, numerous half-life extension techniques have been thoroughly investigated and have been shown to be successful in producing long-acting therapeutics with enhanced pharmacokinetic and pharmacodynamic properties [Tan et al, 2018]. The structural modification of peptides through the reconstruction of the main chain and the adjustment of side chains [Xie et al, 2020] could create analogues, which could exponentially increase gastrointestinal half-lives, improve gut stability and pharmacokinetics. [Braga et al, 2021; Zaman et al, 2019].
3.3.1 Substitution with D-amino acids: As an alternative to improve absorption and intracellular stability, D-chirality amino acids can be included into ACPs [Vaithiyanathan et al, 2021]. D-amino acids are less common in nature and are often unrecognized by many proteases. They are therefore more stable and less immunogenic [Evans, et al, 2020]. Empirical results show that D-chirality peptides are 50 times more stable than their L-chirality counterparts [Vaithiyanathan et al, 2021]. In a study, the antitumor effect of two cationic peptides RDP215 and the D-amino acid variant 9D-RDP215 against Human Melanoma A375 and Glioblastoma LN-229 cells were investigated. D-amino acids in the presence of serum were found to stabilize proteins in ex vivo stability investigations by SDS-PAGE and in vitro with melanoma A375, which was further verified in 2D and 3D in vitro research with glioblastoma LN-229. In LN-229 spheroids, 9D-RDP215 significantly increased levels of cell death after passing a BBB model [Maxian et al, 2021].

3.3.2 Substitution with β-Amino acid: ACPs can gain in vivo metabolic stability and efficacy when one or more β-amino acids are incorporated into them. β-Amino acids are molecules widely found in plants, microbes and marine organisms. They are rare in mammals hence the decreased recognition by human proteases compared to α-amino acid analogues [Evans et al, 2020]. When compared to the unmodified peptide in studies using in vitro tumour cell cultures, it was discovered that the incorporation of a β-alanine-β-alanine (βAla-βAla) linker into a human tumour targeting bombesin peptide resulted in a two-fold increase in metabolic stability against proteolytic degradation and no decrease in receptor affinity. The analogue with the βAla–βAla linker, however, demonstrated lower metabolic stability when the investigations were conducted in human plasma, with a half-life of 10 h as opposed to 16 h for the unmodified analogue [Garayoa et al, 2020].

3.3.3 Substitution with unnatural amino acids (UAAs): This can stabilize the secondary structure of ACPs [Barber, et al, 2022]. UAAs can be integrated into linear or cyclic peptides and they demonstrate biological activity as free acids, making them valuable building blocks in the production of a variety of drugs. Natural amino acids or related chemicals can be chemically modified to produce a UAA [Narancic, et al, 2019]. In a study, the anticancer activity of UAA-containing tripeptides has been evaluated [Nahhas, et al, 2018]. Cytotoxicity tests revealed that naphthalene-containing tripeptides with α-aminoisobutyric acid (U) between the two phenylalanine groups (NapFUF) specifically reduced viability of melanoma cancer cells at 0.25 wt% over the course of a 24-hour period compared to controls (no additives). This finding suggests that that naphthalene-tripeptides containing α -aminoisobutyric acid have potential benefits in the treatment of cancer [Nahhas, et al, 2018]. Also, it has been discovered that an extracellular GAPDH secreted in humans inhibit the growth of cancer cells and a minimal peptide sequence was associated with this activity. Further investigations revealed that when the residues of the peptide were substituted with UAAs, a stronger activity was demonstrated [Yoshida et al, 2022].

3.3.4 N– & C- terminal modification: Terminal modifications such as N-acetylation and/or C-amidation can enhance the stability of peptides by preventing their hydrolysis by exopeptidases causing greater bioavailability and penetration of membrane [Gianfranceschi et al, 2018]. In a study, the MTT-assay was used to analyze the effects of N-terminal acetylation and C-terminal amidation on the cytotoxic properties of the ß-hairpin antimicrobial peptide tachyplesin I. According to the results, modified Tachyplesin I was more hazardous to both tumour and healthy human cells. The peptide with C- and N-terminal modifications is resistant to proteolytic breakdown in fresh human serum, in contrast to Tachyplesin I that has not been changed. As a result of C- and N-terminal modifications, Tachyplesin I's stronger cytotoxic activity and improved ADME properties, make it more appealing model of an anticancer drug [Kuzmin et al, 2017].

3.3.5 N-methylation: Also known as N-alkylation, N-methylation can also be used to modify the structures of ACPs. This technique entails adding N-methyl substituents to one or more NH groups in a peptide backbone. Compared to their unmodified analogues, N-Methylation can result in a number of advantages, such as increased biological activity, membrane permeability, and protease resistance [Linde et al, 2008]. This happens as a result of the N-methyl group's influence on the side chains of residues near the N-methyl amino acids as well as the peptide backbone's structural flexibility [Evans et al, 2020]. The modulation of Ubiquitin (Ub) chains is a potential target in the treatment of cancers but the discovery of peptides to bind with them remains a challenge due to in vivo instability. A recent study reports the identification of short, non-proteinogenic cyclic peptides that can strongly bind Lys48-linked Ub chains and are rich in non-canonical properties such N-methylation. These peptides simultaneously activate three Lys48-linked Ub units, inhibit deubiquitinases and the proteasome, trigger apoptosis in vitro, and inhibit tumour growth in vivo [Rogers, et al, 2021].

3.3.6 Cyclization has a significant impact on the conformational stability of peptides. It increases rigidity and prevents proteolytic degradation [Li et al, 2018]. This includes head to tail cyclization which involves stapling the N and C termini of the peptide backbone; head to side chain; tail to side chain; or side chain to side chain [Evans, et al, 2020]. Peptide has the potential to inhibit PD-1/PD-L1 immune checkpoint but their use is constrained by undesirable physicochemical properties and proteolytic stability profiles [Fetse et al, 2022]. Studies have shown that cyclization enhances the stability and biological activity of linear peptides. In a recent study, a method for finding cyclic anti-PD-L1 peptides with enhanced bioactivity called macrocyclization scanning was employed. The cyclic peptides showed considerable in vivo anti-tumor activity as well as up to a 34-fold improvement in PD-1/PD-L1 blocking efficacy. The findings show that macrocyclization scanning can be used to enhance the anti-PD-L1 linear peptide's serum stability and bioactivity [Fetse et al, 2022].

3.3.7 Conjugation: Peptide drugs often have molecular weights below the renal filtration cut-off of 50 kDa, allowing for quick excretion by the kidney [Kontermann, 2016]. Renal filtration and clearance can be reduced by conjugating peptides with bigger molecules which will increase steric hindrance around cleavage sites and lengthen circulatory half-lives [Li et al, 2018]. Some of the macromolecules that can be used to prolong the peptide half-lives include HSA (human serum albumin), Fc (crystallizable immunoglobulin fragment), XTEN (extended recombinant polypeptide), and PEG (polyethylene glycol) [Strohl, 2015]. For example, one or more polyethylene glycol (PEG) polymer chains can be added to the ACP. PEGylation increases solubility, the molecular mass, and the metabolic stability of the peptide. Consequently, this prolongs the half-life of the peptide [Evans et al, 2020]. Studies have demonstrated that the introduction of PEG into A20FMDV2 peptides labeled with 4-18F-fluorobenzoic acid (18F-FBA) improved the pharmacokinetics and increased uptake in αvβ6-expressing tumors [Hausner et al, 2015].

3.3.8 Others: Chemical modifications such as altering the cysteine residue increased the stability of the TAT peptide and hence improved plasmid distribution [Kumar et al, 2020; Zaman et al, 2019]. Fluorine substituents also have a significant impact on peptides' pharmacokinetics and biological half-lives. Fluorinated amino acids can be used to increase the proteolytic stability of ACPs [Huhmann & Koksch 2018]. Other techniques for increasing peptide stability include glycosylation, palmitoylation, and phosphorylation [Xie et al, 2020].

3.4 Tumor-Homing ability

The absence of screening diagnostics for early detection and the lack of tumor-specific drug delivery systems are the main causes of cancer high mortality rate. The majority of currently available anticancer drugs are unable to distinguish between malignant and normal cells, which causes systemic toxicity and unfavourable side effects [Gautam et al, 2014]. Over the years, significant progress has been made in identifying peptides that specifically bind to tumour cells and tumour vasculature in order to address this issue [Gautam et al, 2014]. ACP must have tumor-homing ability and properties that enhance this includes charge and length.

3.4.1: Charge: An effective ACP must be cationic because cancer cells which produce lactate ions in excess are negatively charged [Pérez-Tomás, & Pérez-Guillén, 2020]. It is thought that a net charge differential between neoplastic and non-neoplastic cells at the membrane surface is what largely causes peptide selectivity for cancer cells [Gabernet et al, 2016]. The tumor homing ability of an ACP is be based on the electrostatic interaction with the negatively charged tumour cells. This provides the environment for strong binding and selective disruption of the outer membrane of tumour cells [Scocchi et al., 2016; Lei et al., 2019]. This interaction does not exist with normal cells because they have a neutral charge. The primary mechanism of action of ACPs is to cause cell membrane disintegration or apoptosis by depolarizing the cell membrane, which prevents tumour cells from maintaining a normal osmotic pressure. Consequently, the destroyed membrane of cancer cells leads to massive leakage of the contents of the cytoplasm [Xie et al, 2020]. In human breast cancer cells, ACPs have also been shown to cause mitochondrial apoptosis, the release of Cytochrome C and the trigger of the caspase apoptotic pathway [Xie et al, 2020].

3.4.2: Size/length: the size of a molecule determines the speed at which it is transported across cell membranes [Yang et al, 2015]. Few peptides have length greater than 50 residues [Manavalan, et al, 2017] and specifically a tumor-homing ACP range from 5 to 30 amino acids in length [Lingasamy et al., 2020]. Distinctly, therapeutic peptides stand between two molecular extremes of proteins (macromolecules) and small molecules, and offer benefits [Craik, et al, 2013] such as high activity, good biocompatibility in vivo, low immunogenicity, ease and speed of synthesis [Norouzi, et al, 2022]. The design ACPs can also be easily modified because of their size [Kondo et al, 2021].

3.5: Cell-penetrating ability: Beyond have membranolytic activity, ACPs could also be cell-penetrating peptides (CPP). Since they can pass through cell membranes, CPPs, are frequently employed to deliver proteins, plasmid DNA, RNA, oligonucleotides, liposomes, and anti-cancer drugs into intracellular targets [Borrelli, et al, 2018]. For instance, it has been proven that doxorubicin's improved anticancer activity is mediated by the human-derived cell-penetrating peptide dNP2. The peptide-drug conjugate demonstrated enhanced DNA damage capability, increased apoptosis, and tumour inhibition than the unconjugated doxorubicin [Xiang et al, 2018]. Also, researchers created a weakly acidic, pH-activatable cell-penetrating peptide dimer (LH2) with histidine residues that can enter cells even at concentrations as low as a few tens of nanomolar. Through the creation of non-covalent complexes or covalent conjugates, LH2 efficiently transported paclitaxel into triple-negative breast cancer cells, MDA-MB-231 [Nam et al, 2021].

3.5.1 Helicity: Helicity plays a major role in enhancing cell penetration. Through mimicking the transmembrane (alpha helix) domains [Mahendran et al, 2017] in nature, it is possible to improve penetration efficiency and internalization kinetics [Kalafatovic & Giralt, 2017]. It has been demonstrated that the hydrophobicity of the peptides' nonpolar face correlates with their helicity. Reversed-phase high performance liquid chromatography temperature profiling revealed that the propensity of peptides to self-associate in an aqueous environment had a significant impact on their anticancer activity. The anticancer activity depends heavily on the hydrophobicity of peptides [Huang et al, 2011]. Therefore, the hydrophobic composition is a key factor in designing ACPs for cell penetration. The incorporation of D-chirality residues into peptide sequences have also been shown to cause a threefold increase in uptake as compared with the L-chirality amino acids with internalization through endocytosis and direct penetration [Vaithiyanathan et al, 2021].

3.5.2 Prenylation: Peptide prenylation can also enhance cell-penetrating ability. It involves the transfer of either a Prenyl (farnesyl or a geranylgeranyl) moiety to C-terminal cysteine(s) of the target protein. This modification has been known to enhance attachment to cell membrane and protein-protein interaction [Casey & Seabra, 1996]. Potential anticancer drugs based on naturally occurring prenylated indoles have been suggested and as such researchers incorporated prenylated indoles into primary sequences to create a novel ACP [Offerman et al, 2017]. Located close to the C-terminal of a pentapeptide, the indole ring on the tryptophan residue was N-tert-prenylated. Upon in vitro screening, it was revealed that the modification enhanced significant cytotoxicity against H69 and DMS79 Small Cell Lung Cancer (SCLC) cell lines as compared with the unaltered pentapeptide or Substance P antagonist G (SPG), a well-known anti-SCLC agent [Offerman et al, 2017].

Conclusion

ACPs have shown great promise as an alternative to small molecule chemotherapy in the treatment of cancers. In the de novo design of safe and efficacious ACPs, properties such as druggability, allergenicity and toxicity, stability, tumor-homing ability, and cell-penetrating ability must be considered. Though these properties can be predicted using in silico tools, further evaluation using in vitro and in vivo methods are also required.

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