PEPTIDE DRUG CONJUGATES: A NEW HOPE TO IMPROVE CANCER MANEGMENT

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***Abstract*- Cancer remains the leading cause of death worldwide despite advances in treatment options for patients. As such, safe and effective therapeutics are required. Short peptides provide advantages to be used in cancer management due to their unique properties, amazing versatility, and progress in biotechnology to overcome peptide limitations. Several appealing peptide- based therapeutic strategies have been developed. Here, we provide an overview of peptide conjugates, the better equivalents of antibody-drug conjugates, as the next generation of drugs for required precise targeting, enhanced cellular permeability, improved drug selectivity, and reduced toxicity for the efficient treatment of cancers. We discuss the basic components of drug conjugates and their release action, including the release of cytotoxins from the linker. We also present peptide-drug conjugates under different stages of clinical development as well as regulatory and other challenges.**

***Keywords*: short peptides; peptide-drug conjugates; drug delivery; cancer; Linkers; nanotechnology.**

INTRODUCTION

Cancer remains one of the major diseases with a high mortality rate despite advances in enhanced diagnostics and treatments. In women, the leading cancer is breast cancer, and in men, it is prostate cancer. At the genetic level, cellular DNA is altered, leading to abnormal gene expression patterns. Consequently, the effects of normal genes which control normal cellular functions such as growth and survival of the cell and invasion/ motility, are accentuated. Accumulation of mutations is the main mechanism of alteration. However, non-mutational changes occur through the DNA methylation process, which is recognized as central to the process. Primarily, cancer can be treated by various approaches including surgery, chemotherapy, radiotherapy, and immunotherapy. Chemotherapeutic drugs generally have a low capacity to penetrate the parenchyma of solid tumours, hence, improvements are vital. In addition, the overexpression of cancer associated antigens allows targeted approaches for drugs to be delivered as antibody–drug conjugates or peptide-drug conjugates, with minimal effects on healthy cells. As we know, communication between cells and their cell surface proteins is Required for their survival. In a single organism, numerous cells are connected to form an interactome. The interactome is an extensive network system composed of protein–protein interactions (PPIs) and an array of molecular interactions. These PPIs are necessary for chemical biology and medicinal chemistry, and modulate signaling pathways and the cells' systemic functions. Some cellular processes such as transcription, translation, transduction, and replication are catalyzed by PPIs. In living cells, PPIs and proteins play a role in functional building blocks. Diseases such as cancer, infectious and neurodegenerative diseases, and disturbances n cell homeostasis can be caused by errors in a central node/hub of the PPIs network. For the development of new drugs and novel diagnostics, PPIs are attractive targets and have efficient therapeutic potential Peptides, as constituents of proteins, are promising, safe, and effective anti-cancer therapeutics and are engaged in nearly all biological functions. Short peptides do not induce an undesired autoimmune response; they have high selectivity and specificity, are able to cross cell membranes, and penetrate tumours more efficiently. Herein, applications of peptide-drug conjugates, their basic components, conjugation chemistry release action, and different stages of their clinical development, regulatory procedures, and other challenges are presented.

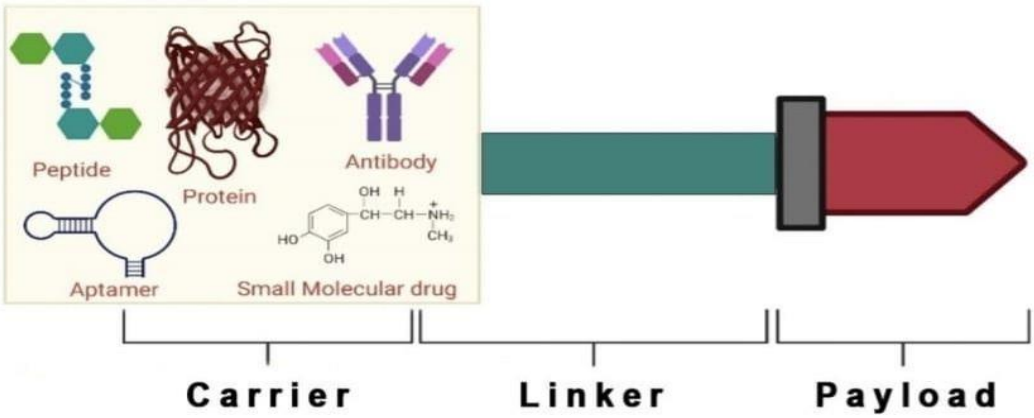
PEPTIDE–DRUG CONJUGATES

In peptide-drug approaches for cancer, it is important to identify a target for cancer cells and a method to target the drug to such cells Peptides facilitate the penetration of drugs into the body. A chemical modification of peptide and protein drugs improves their enzymatic stability and membrane penetration of peptides and proteins. Thus, much
focus has been on tumour-targeting peptides. For example, cancer cells, and the tripeptide arginyl-glycyl-aspartic acid (RGD) express adhesion receptors, αvβ3, and αvβ5 integrins-peptides selectively bind to these integrins, allowing drug delivery. In the parenchyma of the tumour, peptides show limited loading capacity but can deliver drugs, biologics,
viruses, and nanoparticles to blood vessels successfully. While the molecular targets in non-targeted approaches are not clear, natural and synthetic molecules are screened based on their ability to produce a sufficient phenotypic change in cells, including depletion of mitotic and motility defects and initiation of apoptosis. As such, the final hit

compounds are selected based on efficacy to restrict the desired phenotypic properties with minimal side effects.

Currently, peptides are used for several diseases, representing a powerful class of medicines. Short peptides are advantageous over biologics and small molecules, as they can interact with unknown targets, have a straightforward design, are relatively cheaper to synthesize, and have enhanced tissue penetration. Peptides show excellent performance in transporting molecules to desired targets. Thus, such advantages lead to the identification of several naturally occurring and modified peptides, in addition to their combination with other systems, such as peptide–nanoparticles and peptide-drug conjugates. Countless strategies are available for identifying peptides with anticancer activity for targeted and non-targeted approaches. The non-targeted approaches are evaluated from natural sources such as plant extracts, algae, toxins, animal venom, microbes, or screening of synthetic libraries. A targeted approach uses a direct evolution technique for rationally designed peptides for selected targets. Peptide-drug conjugates (PDCs) are composed of different components, which include a homing peptide or device, cytotoxic payload, and a linker that works synergistically to deliver cytotoxins to the targeted receptor on cancer cells (Figure 1). Each component's role and mechanisms of action are important considerations when assembling PDCs.

Figure 1 : Schematic diagram of the components of peptide-drug conjugates



C0MPONENT OF PEPTIDE-DRUG CONJUGATES

Several approaches have been explored for the selective delivery of effector molecules to cancer cells. PDCs are composed of three main components, including homing peptide, linker, and payload (Figure 1)

a) Peptide
b) Linker
c) Payloads

Peptide

The first component of PDCs is the carrier or the homing peptide, which eases tumour targeting. Several biologicals ,including antibodies, proteins, peptides, and small molecules have been investigated in addition to aptamers to facilitate tumour selectivity. The homing peptide is a selected peptide that has a specificity for targeting molecules to specific protein receptors expressed on cancer cells, and often displays high affinity to the target receptor site. Thus, the homing peptide directs the PDC at the desired targeted cell or tissue and limits off-target delivery. The binding affinity of homing peptides is an important feature, and it depends on the secondary structure of homing devices such as random coil, β-sheet, and α-helix. This allows greater binding affinity through enhanced stabilization of the secondary structure of the homing peptide. However, this approach has certain disadvantages, such as chemical instability, degradation by enzymes, and fast renal clearance. However, it is possible to gain control over such types of drawbacks by developing modifications of the peptide such as cyclization techniques or non-natural amino acids. Cyclization is achieved either via side chain to side chain, head-tail chain to side chain, or head-to-tail modifications, which are more stable and active compared to their linear counterparts, as seen in various studies. To produce peptide-drug conjugates, several homing peptides have been used (Table:1)

Table 1. Homing peptides used for the development of Peptide-drug-conjugates

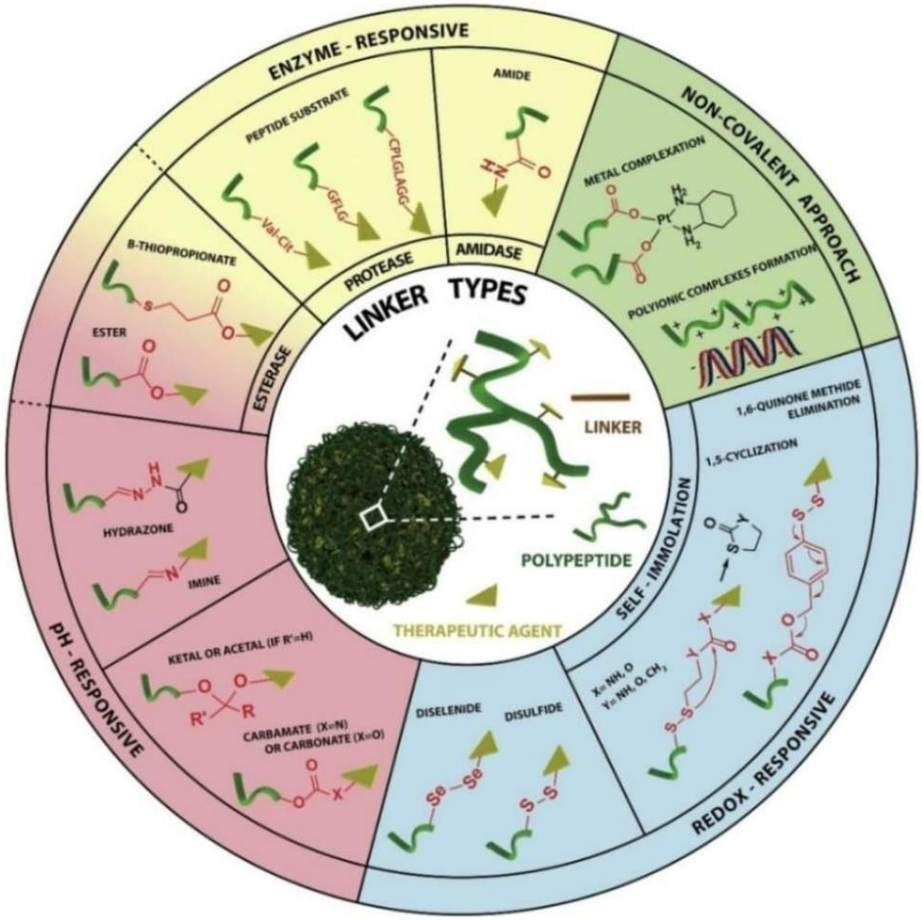
Homing Peptide	**Receptor Site**
Somatostatin	Somatostatin receptor
Octreotide	Somatostatin receptor 2/5 (SSTR2/5)
GE11	Epidermal growth factor receptor (EGFR): ErbB1
Epidermal growth factor (EGF)	Epidermal growth factor receptor (EGFR): HER1, HER2, HER3, HER4

Peptides can be used as therapeutic agents and as drug carriers, but the criteria for action and selection of peptides are quite different. Herein, we discuss all the aspects of peptides. They are used in biotechnology for diagnostic and therapeutic purposes As an example, calcitonin gene-related peptide is used for migraines, bombesin is used in prostate cancer, somatropin pegol is used in growth hormone deficiency, and BPI3016 is used in type-2 diabetes as a therapeutic agent. In recent years, peptides have demonstrate improved binding affinity and specificity for challenging binding interfaces due to their smaller size and balance of conformational rigidity and flexibility.

Linker

In peptide-drug conjugations, linkers play a crucial role by linking small drug molecules to peptides and maintaining the structural integrity of PDCs. Based on the mechanisms of PDC, the linker should be selected based on its stability within the circulation to prevent non-specific and premature releasing of the drug at receptor sites, which may result in adverse effects. As such, several conditions are considered when determining which linker to use including pH, enzyme responsiveness, redox responsiveness, and non-covalent linkers (Figure 2).

Figure 2: Different types of polypeptide-drug linkers commonly used in the rational design of poly-Peptide drug Conjugates



Payloads

The main and third component of PDCs is the drug itself, which can persuade a variety of biological functions. However, in the treatment of cancer, radionuclides and cytotoxic molecules are widely used.

a) Chemotherapeutic Agents:

Based on the general mechanism of action, cytotoxic agents are classified as the first group of cytotoxic agents which interfere with the DNA protein complexes/cellular DNA and lead to apoptosis. Anthracyclines such as daunorubicin (DAU) or doxorubicin (DOX) and Camptothecin (CPT) are widely used in PDCs. The second group of agents act by inhibiting the bio -synthesis of DNA. It is a class of antimetabolites. Folate derivative methotrexate (MTX), nucleoside Analog of deoxycytidine, and gemcitabine are widely used. The third group of agents act on microtubules with anti-mitotic abilities. Vinka alkaloid Analog and Paclitaxel (PTX) belong to this class of agents. There are thousands of antitumour agents available and a wide range of cytotoxic drugs on the market, and each of them come with different limitations including poor pharmacokinetic properties and generation of toxicity to nearby healthy cells

whilst targeting an affected one. To overcome these unwanted
effects, cytotoxins are attached to peptides with the reduced dose, since peptides provide a specific targeted delivery, and hence the greater proportion of the drug being reached to the specific target site.

b) Radionuclides

Radionuclides are the second most important type of payload in PDCs. It can be used for two main purposes, that of diagnosis or in therapeutic settings.

Delivery of Peptide Drug Conjugates to the Desired Targeted Tissue

When delivering drugs to cancer cells, the primary goal is to minimize their impact on healthy tissues and ensure that a sufficient amount of the drug reaches the target. This is achieved using two main approaches, both of which aim to alter the pharmacokinetic properties of the drug i) use of a delivery vehicle such as nanoparticles, which encapsulate the drug and can dictate its biodistribution through its physicochemical properties, and ii) to covalently modify the drug with a small moiety that masks the drug's bioactivity or limits its pharmacokinetic properties. Using peptides, PDCs can have a great degree of functionality, as they can be controlled physiochemically and actively target a particular receptor on cancer cells. The peptides in PDCs are generally short in length and biodegradable, to avoid unwanted immune reactions. By combining different amino acids, a wide variety of PDCs are easily prepared. The sequence controls both the hydrophobicity and the ionization of the conjugate, which affects bioavailability in vitro and in vivo. The physiological action of PDCs depends on several factors including the homing peptide and the linker. Cancer-targeted therapeutic approaches rely on cell surface receptors because they provide specific targeting properties for tumours. To achieve sufficient selectivity, cell surface receptor overexpression is required. It is usually desirable to have a normal cell-to-tumour expression ratio above 1:3. As discussed, the linker prevents premature release or unwanted release of cytotoxins that can be responsible for the adverse effects. Thus, it is important to gain control over the release of cytotoxins from the linker. A cleavable linker should be cleaved in the presence of a suitable pH or enzymes Two alternates are available; in the first approach, PDCs follow a similar kind of action to that of antibody-drug conjugates such as the first internalization and then release of a cytotoxin from the linker by cleavage, while the second approach includes cleavage release of a cytotoxin outside the cell followed by internalization. Homing peptides play an important role in targeting the PDCs at specific receptors as they can be a non-penetrating or penetrating peptide. Generally, non-penetrating homing peptides bind with the targeted receptor site and initiate receptor-mediated internalization and endocytosis. PDCs are transported from early endosomes to late endosomes and enter lysosomes, where the pH is low and which contain specific enzymes, where the PDC is cleaved and releases the cytotoxin. As a result, peptide-binding receptors are potential drug carriers with tumour-specific properties due to the properties associated with their activating peptide ligands. Additionally, small molecules or antibodies can also be used to target peptide-binding receptors. As such, the drug compound can be covalently linked to the receptor-binding molecule, making it possible to deliver the drug in a targeted manner. Furthermore, the main demerits are fast renal clearance and poor stability of PDCs in circulation. To overcome these drawbacks, gold nanoparticles and conjugation to antibody Fc or albumin have been used together with the PDCs due to their longer circulation half-life, reliable physicochemical properties, and safety properties which enhance the overall stability of PDCs. One of the greatest advantages of PDCs is that they may overcome/bypass the development of drug resistance in cancer cells. As such, branched cell penetration of peptide-drug conjugates have been studied to overcome drug resistance. Many valuable anti-cancer drugs have undesirable side effects in the current clinical setting, including drug resistance and inefficient cellular uptake. There are several strategies for overcoming limitations in therapeutic research, such as the construction of highaffinity multivalent ligands for drug delivery or selective tumour targeting of chemotherapeutics. Cell-penetrating peptides are particularly effective delivery vehicles, and conjugates of these peptides with anti-cancer medicines have been used to improve cellular uptake They concluded that in comparison to their linear analogue and doxorubicin, new branched doxorubicin–oligoarginine conjugates demonstrated superior efficacy against wild-type and resistant neuroblastoma tumor cell lines, For the first time, these novel conjugates confirm the effect of high local

concentrations on cellular uptake and cytotoxicity in resistant cells. Radiotherapy is mostly used as a treatment of cancer, but it increases normal tissue damage, so it is conjugated with peptides. Peptides linked to radio sensitizing monomethyl auristatin E selectively stimulates CD8+ T cells and are reliant on long-term tumour control and immunological memory in irradiated cells. Monomethyl auristatin-E sculpts the tumour immune infiltration in conjunction with ionizing radiation to enhance immune checkpoint inhibition. This is how drug resistance is overcome by PDCs, which will be beneficial for treating trimodal cancers. Efficiency of PDCs depends on the targeted receptor, the pathway of receptor-mediated-endocytosis, and intracellular trafficking. For the treatment of cancer, several cancer- specific receptors or markers are targeted by PDCs such as the integrin (αvβ3) receptor for ovarian cancer, EGFR receptor and MUC1 (CD227) for lung, breast, bladder, and ovarian cancer, NPY(Hy1R) receptor for breast cancer and Ewing sarcoma, and MC1R receptor for melanoma .By the targeted drug delivery approach, side-effects can be reduced, and the efficacy of the drug can be increased. Trafficking of extracellular macromolecules into cells (endocytosis) and across cells is facilitated by receptor-mediated transport mechanisms. During this process, ligands bind to specific cell surface receptors, cluster together within endocytic vesicles, and sort into their respective vesicles. Internalization and trafficking of CSPG-bound recombinant VAR2CSA lectins are examples and used in cancer treatment.

Application

PDCs have a broad range of applications. They can be applied against viruses such as SARS-CoV-2 or multi resistant bacteria. PDCs also have relevance in the development of efficient vaccines. CoVac501, a self-adjuvanting peptide vaccine conjugated to a toll like receptor (TLR)-7 agonist is a promising therapeutic against SARSCoV-2 with antifungal applications Likewise, in cancer, a self Adjuvating multicomponent vaccine combining Perglycosylated MUC1 peptide and TLR2 agonist Pam3CysSer shows strong antibody generation in animal models. Moving forward, peptide conjugates with a fluorescent dye can be used in diagnostic applications i.e., cancer imaging. In addition, poly -anionic peptides conjugated with anti-inflammatory drugs are delivered into bones and other tumour cells. Peptide conjugates acting on the central nervous system or in gene delivery are promising.

I) Anti-Cancer Therapy :

In the context of applications of conjugated peptides for anticancer therapy, apoptosis and tumour accumulation-related peptides as well as the role of peptide-based nanotechnology, are discussed.

II) Tumour Accumulation :

Diverse techniques for targeting tumours with peptides have been introduced. For example, non-RGD (arginine-glycineaspartate) disulfide (SS)-bridged cyclo-peptide (ALOS-4) has specificity for integrin avβ3, overexpressed on several cancers including human metastatic melanoma. ALOS-4 conjugated with a
topoisomerase I inhibitor, Camptothecin revealed increased cytotoxicity in human metastatic melanoma cells and decreased cytotoxicity in normal cells. The tetra-branched peptide NT4 conjugated with paclitaxel binds to tumour membrane sulphated glycosaminoglycans with strong selectivity showing more effective tumour regression. Dimeric 123B9 peptide, targeting EphA2 conjugated with paclitaxel inhibits lung metastasis in breast cancer models. A protein-G-derived albumin-binding domain (ABD) conjugated with doxorubicin via a pH-sensitive linker demonstrates a longer half-life in the plasma and four times
higher accumulation within the tumour. Albumin-binding peptide (DICLPRWGCLW)- based bioconjugates are stable complexes for tumour-targeting effect.

III) Cancer Immunotherapy :

The treatment of cancer can be possible by controlling the function of immune cells. Programmed cell death ligand (PD-L1) is overexpressed on several cancer cells. Interaction with program - mmed cell death protein 1 (PD-1; CD279), overexpressed on activate T cells, enables immune evasion of cancer cells.
Inhibiting PD-1/PDL1 interactions is a promising therapeutic approach.

Challenges for the Delivery of Peptide-Drug Conjugates: The Way Forward

Despite the remarkable progress in the research of PDCs, they still face challenges such as poor stability, rapid renal clearance, and translation of their favourable features into clinical outcomes for patients. PDCs often fail in clinical trials due to difficulty in translating rational drug conjugate designs into effective anti-cancer therapies. The question of how and when PDCs are is released after entering the cell remains open. Thus, PDCs must be designed and synthesized more efficiently. Notably, fluorescent probes (such as fluorogenic with aggregation-induced emission feature) can be incorporated into PDCs to monitor this process. Another problem is the oral administration of peptides as a new strategy for utilizing potential PDCs in human clinical trials. In this context, a new self-assembly method to form Pectindiydroartemis in Hydroxycamptothecin nanoparticles to PDC delivery by the oral route is worth mentioning. It could improve drug loading and release.

Conclusions

Peptide-drug conjugates are a great promise for cancer management, PDCs are a next-generation of targeted therapeutic agents as the equivalent antibody-drug conjugates, offering many advantages such as enhanced tumour penetration, selective delivery of cytotoxic payloads to target cells, improved efficacy, multifunctionality, biodegradability, reduced immunogenicity, and systemic toxicity, simplicity, easy structural modification, and lower costs of synthesis. Nevertheless, there are still challenges to overcome such as stability, bioactivity, and renal clearance. On the other hand, new innovative technologies such as nanotechnology are a promising prospect for potentiating anti- cancer efficacy, with potentially fewer side effects in terms of PDCs, thus driving the development of the field.

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