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Review article

Recent developments in anticancer drug delivery using cell penetrating and tumor targeting peptides

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ABSTRACT

Efficient intracellular trafficking and targeted delivery to the site of action are essential to overcome the current drawbacks of cancer therapeutics. Cell Penetrating Peptides (CPPs) offer the possibility of efficient intracellular trafficking, and, therefore the development of drug delivery systems using CPPs as cargo carriers is an attractive strategy to address the current drawbacks of cancer therapeutics. Additionally, the possibility of incorporating Tumor Targeting Peptides (TTPs) into the delivery system provides the necessary drug targeting effect. Therefore the conjugation of CPPs and/or TTPs with therapeutics provides a potentially efficient method of improving intracellular drug delivery mechanisms. Peptides used as cargo carriers in DDS have been shown to enhance the cellular uptake of drugs and thereby provide an efficient therapeutic benefit over the drug on its own. After providing a brief overview of various drug targeting approaches, this review focusses on peptides as carriers and targeting moieties in drug-peptide covalent conjugates and summarizes the most recent literature examples where CPPs on their own or CPPs together with TTPs have been conjugated to anticancer drugs such as Doxorubicin, Methotrexate, Paclitaxel, Chlorambucil *etc.* A short section on CPPs used in multicomponent drug delivery systems is also included.

Contents

1. Introduction

Despite significant advances in the development of anticancer drugs during the last decade, cancer still remains one of the leading causes of death globally. Lack of tumor specificity, inefficient drug accumulation in tumors, cancer cell heterogeneity and drug resistance are all factors that contribute to the lack of effectiveness observed in cancer therapeutics and are their major drawbacks. Drug resistance often results from inactivation of the drug *in vivo*, alteration of drug targets and drug efflux. The drugs poorly differentiating between cancer and normal cells is still a major drawback of cancer drugs. This results in undesirable

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side effects, incomplete tumor response, and eventually therapeutic failure [1–4]. The development of site specific drug delivery systems (DDS) and efficient drug targeting approaches that help to overcome the systemic toxicity exhibited by cancer therapeutics is an active area of research in academia and industry [5–9]. Commonly used strategies in drug delivery include self-assembly, PEGylation [10], stimulus sensitivity (such as pH-sensitive and thermosensitive systems) [11], enhanced permeability and retention [12], the use of cell-penetrating moieties [13–16] and the concept of prodrugs [17]. Amongst these strategies, the covalent conjugation of a cytotoxic drug to a carrier molecule has gained popularity. In this review we focus on peptides as carriers and targeting moieties in drug-peptide covalent conjugates. The three key components in DDS are the carrier molecule, the therapeutic and the linker and/or spacer that join the two molecules together (Fig. 1) Most DDS designs are based on biodegradable carrier molecules, to ensure minimal toxicities. A class of molecules known as 'cell penetrating peptides' have been particularly studied during the past two decades as carriers [13–14]. These peptides are highly advantageous as they are biocompatible and the peptide sequence can be modified to fine tune hydrophobicity, affinity, charge, solubility and stability. They can also be readily synthesised in sufficient quantity. The following sections discuss recent advances in the use of two classes of peptides which have been made use of in drug delivery and drug targeting. These are the Cell Penetrating Peptides (CPPs) and the Tumor Targeting Peptides (TTPs). We discuss recent examples from the literature in which these peptides have been used in the field of cancer therapeutics on their own as well as their combined use for drug delivery. The review does not include metal-peptide conjugates in particular, even though certain examples where this approach is intrinsically present, in a broader sense, appears throughout. For a recent review on peptide-metal complexes the reader is referred to [18].

2. A broad overview of drug targeting approaches

This section gives a broad overview of drug targeting approaches other than the use of TTPs. Molecular recognition events such as ligand-receptor or antigen-antibody interactions form the basis of active targeting of drugs to cancer cells. The direct conjugation of targeting molecules to drugs or through the use of delivery systems is often used for targeted delivery of drugs to the site of action. In addition to TTPs which are extensively reviewed in this article, the use of small molecule non-peptidic ligands, vitamins, aptamers, monoclonal antibodies and protein scaffolds are also widely used in drug targeting. Ligand targeted therapy (LTT) often makes use of receptors that are overexpressed on the surfaces of tumor cells. These receptors are often proteins or glycoproteins that have high affinity for and selectivity towards specific ligands that include both peptides and non-peptidic molecules. Receptors overexpressed at the cytoplasmic level are also being exploited for the targeted delivery of chemotherapeutics. Receptor targeted nano-carrier drug delivery systems hold promise in cancer therapy and is an emerging field [19]. Another important aspect in cancer treatment is the ability to target circulating tumor cells (CTCs) since CTC mediated interactions are a major cause of cancer metastasis which results in more than 90% of cancer deaths [20]. The selectivity of sialylated carbohydrate ligands, overexpressed on CTCs, towards the selectin protein was made use of to selectively target and kill CTCs using *E*-selectin functionalized liposomal formulation of doxorubicin and a Halloysite coated microtubule device [21]. A further extension of this work by the same group used circulating leukocytes presented with cancer-specific TNF-related apoptosis inducing ligand (TRAIL) together with *E*-selectin receptor to target and kill CTCs [22]. This approach has shown promise in treating CTCs both *in vitro* in human blood cells as well as in *in vivo* experiments in mice.

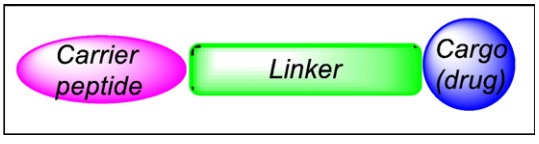

Fig. 1. General scheme of peptide-drug conjugate system.

Different types of nanoparticles are also extensively used for tumor targeting, following both passive and active targeting mechanisms [23–24]. Biodegradable nanoparticles, particularly made from hydrophilic polymers such as polyethylene glycol (PEG) conjugated to high-affinity targeting ligands such as folate have the advantage of delivering large quantities of unmodified drugs in a targeted manner to the tumor. Pegylated and non-pegylated colloidal gold bound to tumor necrosis factor has been shown to preferentially accumulate in tumors [25]. Supermagnetic iron oxide nanoparticles (SPION) have been made use of for targeted aerosol delivery of chemotherapeutics in advanced lung cancer treatments [26].

Multifunctional liposomal nanocarriers, (reviewed in [27] are also used in LTT to enable targeted delivery of anticancer drugs. Antibodies, proteins (transferrin, interlukin *etc.*) small molecules such as folate, estrone or anisamide, carbohydrates, particularly mannose and lactose, chondroitin sulfate or peptides have been used as targeting ligands with liposomal nanocarriers. For example anti-HER2 antibody has been used together with liposomal nanocarriers to specifically target HER2 receptors overexpressed by cancer cells [28]. Conjugation of doxorubicin loaded liposomes to estrone [29–30], folate [31] or tetraiodothyroacetic acid [32] has been found to dramatically increase doxorubicin accumulation in tumors. As stated above, the lack of success of treating metastasis is one of the major causes of cancer deaths. Thus researchers have worked on ways of specifically targeting metastasis. TMTP1 is a metastasis-specific peptide that recognises metastases of various cancers [33]. Conjugation of doxorubicin-loaded liposomes to TMTP1 has been shown to possess deeper tumor penetration and apoptotic ability against metastatic breast cancer [34].

3. Peptides in drug delivery and active targeting

As stated in the introduction, two types of peptides have emerged as useful components in DDS. The first amongst these are the CPPs which have the ability to cross the plasma membrane and thereby facilitate the internalization of otherwise impermeable molecules. The second family of peptides are known as Cell Targeting Peptides (CTPs) or more commonly as Tumor Targeting/Tumor Homing Peptides (TTPs). These peptides interact more specifically with receptors which are overexpressed on tumor cells such as the folate receptors [35–37], integrins [38], somatostatin receptors [39–40], transferrin receptors [41] and epidermal growth factor receptors (EGFR) [42]. The naturally occurring peptides somatostatin [43–44], gastrin [45], substance P [46], α-melanocyte stimulating hormone (α-MSH) [47] luteinizing hormone- releasing hormone (LHRH) [48], bombesin [49–50], vasoactive intestinal peptide (VIP) [51–52] and neurotensin (NT) [53–54] have been identified as being specific to receptors overexpressed in tumors, therefore act as ligands for tumor targeting. The ability to specifically deliver therapeutics to tumor cells decreases unwanted side effects, and increases the therapeutic window [55].

Specific differences between cancer and normal cells are made use of by peptides for targeted delivery to cancer cells. As stated above, cell surface receptors which are overexpressed on cancer cells have been particularly exploited for active targeting using peptide ligand with selectivity and affinity towards these receptors. The possibility of using ligands that specifically bind to these receptor proteins provided a major boost to active targeting. As stated below, the phage display technique has emerged as a powerful method for the discovery of high affinity ligands, particularly peptide ligands that can be overexpressed on the surface of the phages. Even though monoclonal antibody targeting of cancer cell surface receptors is widely used in cancer treatment, the large size of the antibody as well as its non-specific binding are limiting