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Membrane interactions and cell selectivity of amphiphilic anticancer peptides

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Following considerable research efforts on antimicrobial effects by cationic and amphiphilic peptides during the last couple of decades, increasing focus has recently been placed on additional host defense and other biological functions by such peptides, such as anti-inflammatory and anticancer effects. Regarding the latter, it has been increasingly understood that amphiphilic peptides present interesting opportunities not only for reaching selective cancer cell toxicity, but also for promoting uptake of other anticancer therapeutics and of nanopariculate delivery systems containing such drugs. While there is an emerging understanding of the direct antimicrobial function of amphiphilic peptides through bacterial membrane destabilization, the mechanisms underlying their anticancer effects remain less clear. Here, we therefore provide a brief overview on factors affecting toxicity of amphiphilic peptides against tumor and non-malignant cells, and also describe how such peptides can be combined with conjugation moieties or drug delivery systems for increased anticancer effects.

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1. Introduction

Treatment of cancer has seen considerable progress during the last decade through the development of several novel types of therapeutics, including therapeutic antibodies [1] and CAR-T cell therapies [2]. As a result of this, treatment outcome has been improved for a range of tumor types. Despite these considerable advances, however, cancer remains one of the leading causes of death. Reasons for this include insufficient tumor uptake, heterogeneity of cancer cells, as well as numerous pathways for chemoresistance development, including, *e.g.*, drug inactivation, target modifications, and efflux up-regulation. As a result of this, DNA-alkylating agents, hormone agonists/antagonists, and other cancer therapeutics frequently display insufficient selectivity and resulting side-effects. In addition, many such drugs address rapidly dividing cells, resulting in toxic effects also against fast-dividing non-malignant cells, as seen, *e.g.*, from the regular occurrence of decreased blood cell production, digestive tract inflammation, and hair loss due to off-target effects on bone marrow, gastrointestinal tract, and hair follicle cells, respectively [3]. Considering this, specific targeting of cancer cells remains a key issue in cancer research. Strategies for reaching such selective targeting of cancer therapeutics and/or delivery systems include antibody-antigen and ligand-receptor pairs, as well as vitamins, aptamers, and protein scaffolds [4]. In addition, tumor targeting (homing) peptides may allow specific interactions with receptors over-expressed in tumor cells, including, *e.*

g., integrins and somatostatin, folate, epidermal growth factor, or transferrin receptors [5*].

In addition to cancer-targeting through such receptors, cationic amphiphilic peptides provide more general potential for targeting of cancer cells [3]. Such peptides have attracted considerable attention in both academic research and industrial development during the last decade, primarily in the area of antimicrobials. Motivated by increasing resistance development against conventional antibiotics, considerable attention has been placed on antimicrobial peptides (AMPs), which constitute a first line of defense in host defense, as novel antibiotics. Since they act by direct membrane lysis, AMPs cause broad-spectrum antimicrobial effects, for optimized AMPs also against antibiotics-resistant pathogens [6–8]. In addition to such direct antimicrobial effects, some AMPs display additional host defense functions, notably anti-inflammatory and immune modulating effects, of interest, *e.g.*, for preventing the development of sepsis [9,10]. In addition, some AMPs and other cationic amphiphilic peptides display anticancer effects, thought to result from the destabilization of either the outer membrane of cancer cells or of mitochondria membranes in such cells [3,4,11,12]. Key for successful development of cationic amphiphilic peptides into therapeutics is therefore the selectivity of peptide-induced membrane destabilization, so that membranes of cancer cells are destabilized, but not those of non-malignant cells.

Due to their destabilization of the outer membrane of cancer cells, amphiphilic peptides may facilitate cell internalization also of other cytotoxic drugs. Analogous effects may be obtained for nanoparticulate drug delivery systems, thus opening the door for additive or even synergistic effects, and potentially also for multiple modes of actions in combination treatments. For example, doxorubicin displays broad-spectrum antitumor effects. As it is taken up by tumor cells only by passive diffusion, the overall fraction of tumors responding to doxorubicin is quite low (≈30%) [13,14]. Therefore, membrane-destabilizing peptides may facilitate cell internalization of doxorubicin, thus contributing to enhanced anticancer activities. Addressing this, Duong et al. investigated anticancer effects of GRR10W4 (GRRPRPRPRPWWWW) on melanoma, as well the ability of this peptide to increase cell uptake of cargo molecules. GRR10W4 was shown to bind to melanoma cells with a much higher affinity than to non-malignant cells, such as fibroblasts and keratinocytes. In addition, GRR10W4, but not the non-tagged GRR10 (GRRPRPRPRP), was found to facilitate uptake of both doxorubicin and nanopariculate drug delivery systems in melanoma cells, thus mirroring the higher peptide uptake in such cells (Fig. 1) [15*]. Similarly, Zhao et al. found that the combination of doxorubicin, as well as its isomer epirubicin, with the HPRP-A1/HPRP-A2 (Ac-FKKLKKLFSKLWNWK-NH_2) resulted in increased *in vitro* activity against different cancer cells, as well as in increased *in vivo* activity in a HeLa xenograft model in BALB/c nude mice [16]. Analogous effects have been reported for 5-fluorouracil and cytarabine combined with cecropin (KWKLFKKIEKVGQNIRDGIIKAGPAVAVVGQATQIAK-NH_2) [17] and for doxorubicin combined with OLP-1 [18]. Analogously, Sugahara investigated the effects of iRGD (CRGDK/RGPD/EC) conjugated to, or co-administered with, paclitaxel, reporting on synergistic effects in both cases, somewhat more pronounced effects observed on co-administration [19]. Such peptide-induced destabilization of cancer cell membranes provides opportunities not only for drugs/particles with cell internalization efficiency, but also for multi-drug resistant cells displaying up-regulated efflux.

Considerable attention has been placed on cationic peptides as so-called cell-penetrating peptides. Such peptides have been investigated regarding their ability to incorporate cargo molecules in a wide range of cells, not only cancer cells. However, since cell internalization is a prerequisite for most anticancer therapeutics, since transporters for active drug internalization are frequently not expressed, and since efflux pumps frequently transport internalized drug back to the extracellular space, peptides able to pass the plasma membrane of cancer cells offer interesting opportunities for cell internalization of drugs and drug delivery systems [3,4]. Although cell-penetrating peptides vary in composition and structure, they are net positively charged, generally also possessing an amphiphilic character, some frequently investigated samples being penetratin (RQIKIWFQNRRMKWKK-NH_2), TAT (GRKKRRQRRRPQ), and various other arginine-rich peptides. Although the capacity of cell-penetrating peptides to facilitate cell internalization has been extensively demonstrated, there is still some controversy regarding the mechanisms by which this is achieved. Thus, studies have been reported to support both membrane disruption, transmembrane translocation without permanent membrane damage, as well as various active phagocytotic pathways for cell internalization [20–23]. Although there is clearly an overlap between cell-penetrating peptides and anticancer peptides discussed in the present overview, focus will be placed primarily on peptides interacting with, and destabilizing, plasma and other cell membranes in cancer cells. Having said that, it should be noted that anticancer peptides may operate in concert with more specific biochemical pathways. For example, Rouslathi demonstrated peptide-induced activation of endocytic pathways related to macropinocytosis through a process involving binding to a tumor-specific primary receptor, followed by a proteolytic cleavage, and subsequent binding to a second receptor, neuropilin-1 (or neuropilin-2), which activates the transport pathway [5*]. Also illustrating the multifunctionality of anticancer peptides, Figueira et al. demonstrated the defensin PvD [1] to potently damage breast tumor cells, yet not affecting non-malignant breast and brain cells [24*]. In parallel, PvD [1] was found to internalize into cancer cells but to localize in the membrane of non-malignant cells, with no significant detrimental effects on the structure and biomechanical properties of the latter. In addition to this, PvD [1] reduces adhesion of breast cancer cells to human brain endothelial cells, thus providing a dual action, which could potentially be of