Anticancer Activities of Natural and Synthetic Peptides

9

A. L. Hilchie, D. W. Hoskin,
and M. R. Power Coombs

Abstract

Anticancer peptides (ACPs) are cationic amphipathic peptides that bind to and kill cancer cells either by a direct- or indirect-acting mechanism. ACPs provide a novel treatment strategy, and selected ACPs are currently in phase I clinical trials to examine their safety and overall benefit in cancer patients. Increasing the selectivity of ACPs is important so that these peptides kill cancer cells without harming normal cells. Peptide sequence modifications may help to improve ACP selectivity. ACPs also have immune-modulatory effects, including the release of danger signals from dying cancer cells, induction of chemokine genes, increasing T-cell immune responses, and inhibiting T regulatory cells. These effects ultimately increase the potential for an effective anticancer immune response that may contribute to long-term benefits and increased patient survival. Packaging ACPs in nanoparticles or fusogenic liposomes may be beneficial for increasing ACP half-life and enhancing the delivery of ACPs to tumor target cells. Additionally, engineering ACP-producing oncolytic viruses may be an effective future treatment strategy. Overall research in this area has been slow to progress, but with ongoing ACP-based clinical trials, the potential for ACPs in cancer treatments is closer to being realized. The integration of basic research with computer modeling of ACPs is predicted to substantially advance this field of research.

Keywords

Anticancer peptides · Cytotoxicity · Immune modulation · Nanoparticles · Selectivity · Therapeutic

A. L. Hilchie
Department of Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia, Canada

D. W. Hoskin (✉)
Department of Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia, Canada

Department of Pathology, Dalhousie University, Halifax, Nova Scotia, Canada
e-mail: d.w.hoskin@dal.ca

M. R. Power Coombs
Department of Biology, Acadia University, Wolfville, Nova Scotia, Canada

9.1 Introduction

9.1.1 Anticancer Therapies and the Need for Alternative Treatment Strategies

Despite decades of research and progress in the field of cancer therapy, conventional chemotherapy remains the most commonly used treatment modality for most cancers. Chemotherapy functions by indiscriminately killing rapidly dividing

K. Matsuzaki (ed.), *Antimicrobial Peptides*, Advances in Experimental Medicine and Biology 1117,
https://doi.org/10.1007/978-981-13-3588-4_9

cells. As a consequence of this mechanism of action, chemotherapy cannot discriminate normal proliferating cells from cancer cells, and as a result, it is unable to target indolent or dormant cancers (Donnelly 2004; Naumov et al. 2003). Furthermore, the acquisition by cancer cells of a chemo-resistant phenotype further reduces the therapeutic value of chemotherapeutic compounds (Bush and Li 2002). Importantly, certain chemotherapeutic compounds (e.g., cyclophosphamide) are associated with the development of secondary malignancies (Choi et al. 2014). This issue is particularly problematic in pediatric cancers in which secondary malignancies, as well as lifelong consequences of toxicities, represent the most severe long-term complications of chemotherapy (Kebudi and Ozdemir 2017). For example, alkylating agents (e.g., cyclophosphamide and ifosfamide) are commonly used to treat pediatric hematologic malignancies and solid tumors and as preconditioning treatment regimens for hematopoietic stem cell transplantation (Choi et al. 2014). However, these same drugs are known to cause therapy-related acute myelogenous leukemia (Thirman and Larson 1996). To address the many limitations of chemotherapy, significant research efforts over the last decade led to the identification of "targeted therapies" (e.g., trastuzumab) that function by selectively targeting and killing cancer cells while sparing normal healthy cells, regardless of their rate of growth. Unfortunately, cancer cell resistance to these targeted therapies was reported shortly after their introduction to the clinic (Nagy et al. 2005).

Researchers and clinicians alike are now recognizing that novel treatment strategies harnessing the power of the immune system may lead to improved clinical outcomes. Indeed, the use of neutralizing antibodies targeting the immune checkpoints cytotoxic T-lymphocyte-associated protein 4 (CTLA-4; e.g., ipilimumab) and programmed cell death protein 1 (PD-1; e.g., pembrolizumab and nivolumab) has enjoyed considerable clinical success (Seidel et al. 2018; Jean et al. 2017; Furue et al. 2018). These therapies are now used as first- and second-line therapies for the treatment of inoperable advanced melanoma and non-small cell lung cancer, respectively. However, these therapies are not without side effects (e.g., severe diarrhea, colitis, inflammation pneumonitis), and patients with advanced disease often do not respond to treatment or relapse thereafter (Seidel et al. 2018; Jean et al. 2017; Furue et al. 2018; Pillai et al. 2018). Collectively, these issues highlight the ongoing need for novel, broad-spectrum anticancer compounds capable of selectively killing cancer cells. Ideally, these new therapies would also harness the power of the immune system by initiating protective antitumor immune responses in patients.

9.1.2 Anticancer Peptides

Cationic anticancer peptides (ACPs) represent a promising alternative to conventional chemotherapy. ACPs are small peptides that contain several cationic and hydrophobic amino acids, giving them an overall positive charge and amphipathic structure (Hoskin and Ramamoorthy 2008). Most ACPs are inherently antimicrobial in nature. In fact, cationic peptides isolated from various organisms were historically assessed for antimicrobial activities and were studied as such prior to their first being described as potent anticancer agents in 1985 (Sheu et al. 1985). In addition to their antimicrobial and anticancer activities, these so-called host defense peptides (HDPs) exhibit many other biological properties, including antiviral (Wang et al. 2008; Bergman et al. 2007), anti-biofilm (Overhage et al. 2008; de la Fuente-Núñez et al. 2012), wound healing (Steinstraesser et al. 2012), anti-parasitic (Couto et al. 2018), adjuvant (Kindrachuk et al. 2009), and immune-modulatory activities (Madera and Hancock 2012; Nijnik et al. 2010) (Fig. 9.1). Peptides are ideal drug candidates due to their low cost of production, the ease with which they can be modified, and relatively high tissue penetration (e.g., compared to antibody-based therapies) (Soman et al. 2009; Richardson et al. 2009; Hilchie et al. 2012, 2013a, 2015).

ACPs are often classified based on the structure that they adopt upon contact with a biological membrane. Three main classes exist, namely,

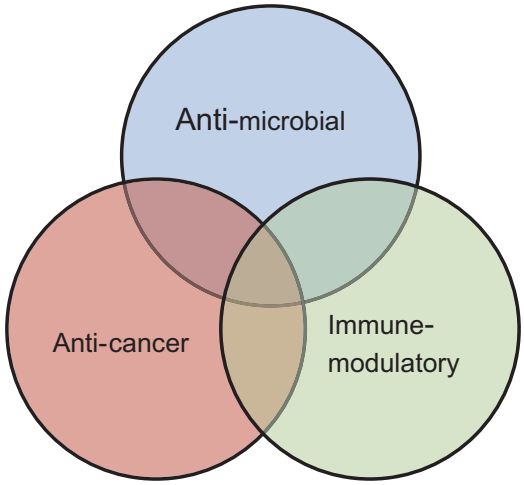

Fig. 9.1 Biological activity of cationic amphipathic peptides. Cationic amphipathic peptides may exhibit any combination of anti-microbial, anti-cancer, or immune-modulatory properties. While many still refer to anti-cancer peptides as cationic antimicrobial peptides, or host defense peptides (i.e., immune-modulatory peptides), it is important to appreciate that these biological activities may be completely independent of each other, and thus should be examined on an individual basis

α-helical (e.g., magainin - Baker et al. 1993; Nguyen et al. 2011), β-sheet (e.g., lactoferricin - Nguyen et al. 2011; Mader et al. 2005), and extended (e.g., LfcinB6 - Richardson et al. 2009; Nguyen et al. 2011). These structures, which are all amphipathic in nature, typically consist of a predominantly cationic face and a hydrophobic face. This is necessary to facilitate peptide interaction with the target cell. ACPs can also be classified on the basis of their mechanism of action, of which two classes exist: direct-acting (i.e., lytic) or indirect-acting (i.e., apoptosis-inducing) (Hilchie and Hoskin 2010), both of which will be discussed in further detail in Sect. 9.2.

9.1.3 Advantages of ACPs Over Conventional Chemotherapy

Due to their unique mechanism of action, ACPs, and particularly direct-acting ACPs (DAAs), have many advantages over conventional chemotherapy. Unlike conventional chemotherapy, many ACPs kill slow-growing as well as multidrug-resistant (MDR) cancer cells (Hilchie et al. 2011; Kim et al. 2003; Johnstone et al. 2000). Several different peptides, including the pleurocidin NRC-03, act as chemosensitizing agents by reducing the EC_{50} of several different chemotherapeutic drugs (Hilchie et al. 2011; Kim et al. 2003; Johnstone et al. 2000; Hui et al. 2002). These chemosensitizing activities suggest that ACPs may work in a synergistic fashion with conventional anticancer drugs. Indeed, we recently showed that the wasp venom peptide mastoparan synergizes with chemotherapeutic compounds both in vitro and in vivo (Hilchie et al. 2016). Many ACPs, including DAAs, destroy primary tumors and their metastases without causing undue harm to normal tissues (Hansel et al. 2007). Moreover, preclinical studies show that DAAs exert antitumor effects when delivered by intratumoral, intraperitoneal, or intravenous injection (Hilchie et al. 2016; Berge et al. 2010; Camilio et al. 2014a). Importantly, the work of others shows that, in addition to their ability to destroy the primary tumor, certain DAAs initiate an antitumor immune response that protects the mouse from tumor rechallenge (Berge et al. 2010; Camilio et al. 2014a). These activities will be discussed in more detail in Sect. 9.3. Furthermore, tumor resistance to DAAs is predicted to be difficult to achieve because DAAs do not rely on unique receptors or a specific signal transduction pathway for their action. Indeed, we investigated cancer cell resistance to DAAs and found that continuous exposure (i.e., more than 1 year) to increasing concentrations of ACPs only generated cancer cells with low-level resistance to lytic peptides (manuscript in preparation). Importantly, these peptide-resistant cancer cells maintained susceptibility to chemotherapeutic drugs and, to our surprise, were unable to establish tumors in immune-deficient mice.

9.1.4 Limitations to the Clinical Use of ACPs

Until recently, the clinical use of ACPs was limited by their high cost of production. However, since their discovery as novel anticancer agents (Sheu et al. 1985), the cost of producing peptides

at high purity (i.e., >95%) by high-performance liquid chromatography (HPLC) has undergone a substantial decline. Moreover, the cost of synthesizing large amounts of good manufacturing practice (GMP)-grade peptide is declining as more and more peptide synthesis companies enter the marketplace. The use of recombinant technology, which is a useful method for synthesizing large amounts of peptides, has to date been very difficult because most ACPs exhibit antimicrobial activities (Greenshields et al. 2008). To address this issue, Ishida et al. recently developed a unique method whereby calmodulin is used as a carrier protein to express several different antimicrobial peptides (Ishida et al. 2016). In this approach, the toxic (i.e., antimicrobial) activities are masked, and the peptide is protected from degradation during peptide expression and purification. Others have taken an alternate approach of identifying truncated forms of the parent peptide that maintain their biological activities (Richardson et al. 2009; Mader et al. 2005). Moreover, identifying combinations of ACPs and chemotherapeutic compounds that synergize in vivo is expected to reduce the dose of each compound that is required for a biological effect, thereby reducing any treatment-related toxicities and overall treatment cost. Collectively, these research endeavors, as well as a competitive marketplace, significantly reduce the financial burden of novel peptide-based therapies.

One of the most significant shortcomings of ACP-based therapies is their toxicity to normal cells at *high* peptide concentrations. Many research groups have attempted to reduce off-target toxicity by adding a targeting sequence to their peptide of choice (Liu et al. 2011; Zitzmann et al. 2002; Leuschner and Hansel 2004). To this end, small targeting moieties that interact with specific cell surface molecules overexpressed on cancer cells are added to the peptide of interest, typically using a glycine-glycine linker. To date, this strategy, which will be discussed in more detail in Sect. 9.5.1, has shown mixed results. It is important to note that this strategy increases the cost of production, as synthesis costs are directly proportional to peptide length, and requires that the tumor cells maintain expression of the receptor with which the targeted peptide interacts. As an alternative strategy, amino acid substitution has been used to reduce peptide toxicity to normal cells (Dennison et al. 2006; Yang et al. 2003; Eliassen et al. 2003). This approach typically involves modifying simple peptide characteristics, such as charge and/or hydrophobicity, as these are known to be required for toxicity to tumor cells; however, the structural basis for selective cancer cell killing by ACPs is still poorly understood. We will further discuss this approach in Sect. 9.5.2.

The in vivo stability of peptides is a significant shortcoming of many ACP-based therapeutics. Unpublished work by the Hancock group suggests that small cationic peptides rapidly distribute to all tissues in the body and possess a half-life of approximately 2 min in the blood (discussed in Hilchie et al. 2013a). While many see this as an issue, others argue that this problem is negated by the speed with which many different ACPs exert their toxic effects to cancer cells and that, by limiting peptide half-life, the likelihood of off-target toxicities is also reduced. Nevertheless, there are several reports that peptidomimetics show improved stability in vitro. Moreover, various nanoparticle-based delivery strategies show considerable promise. These strategies will be discussed in further detail in Sects. 9.4.1 and 9.4.2.

In our opinion, the biggest issue facing ACP-based therapies is the loss of momentum that this field of research is experiencing. Time and time again, researchers identify new ACPs and describe their mechanism of action and perhaps their spectrum of activity (i.e., which cancer cell types are susceptible to peptide-mediated killing); however, there is little follow-up work. Thus, with few exceptions, little has been done to thoughtfully address the shortcomings of ACP-based therapies. Here, our aim is to describe the anticancer potential of these molecules and their mechanism(s) of action and discuss ways in which momentum can be regained in an otherwise promising field of research.

9.2 Direct-Acting Versus Indirect-Acting ACPs

ACPs are classified as direct- or indirect-acting based on their mechanism of action (Hilchie and Hoskin 2010). DAAs bind to and kill cancer cells by causing irreparable membrane damage followed by cell lysis (Fig. 9.2). In contrast, exposure to indirect-acting ACPs results in cell death by apoptosis, which occurs in the absence of extensive membrane damage.

DAAs do not require access to the cytosol in order to kill the cell. As a consequence of this, DAAs have many advantages over conventional chemotherapy (see Sect. 9.1.3). DAAs tend to be highly potent and maintain a relatively broad spectrum of activity (i.e., kill a wide variety of cancer cells) in comparison to indirect-acting ACPs. Indirect-acting ACPs tend to be less potent than DAAs, and they often target mitochondria, thereby killing cancer cells by initiating mitochondrial-dependent (i.e., intrinsic) apoptosis (Mader et al. 2005; de Azevedo et al. 2015). While these two mechanisms vary considerably, both are initiated by the selective binding of ACPs to cancer cell membranes.

9.2.1 Factors That Contribute to Selective Peptide Binding to Cancer Cell Membranes

ACPs are thought to selectively bind to cancer cell membranes because of differences in membrane composition (i.e., charge), surface area, transmembrane potential, and membrane fluidity (reviewed in (Hoskin and Ramamoorthy 2008; Hilchie and Hoskin 2010; Mader and Hoskin 2006; Yeaman and Yount 2003; Bhutia and Maiti 2008; Giuliani et al. 2007)). To our knowledge, no study has definitively elucidated the mechanism by which ACPs selectively bind to cancer cell membranes. However, experts agree that membrane composition (i.e., charge) appears to be the most significant factor in this process. Thus, we will limit our discussion to the importance of membrane composition, as the other factors have been reviewed elsewhere (Hoskin and

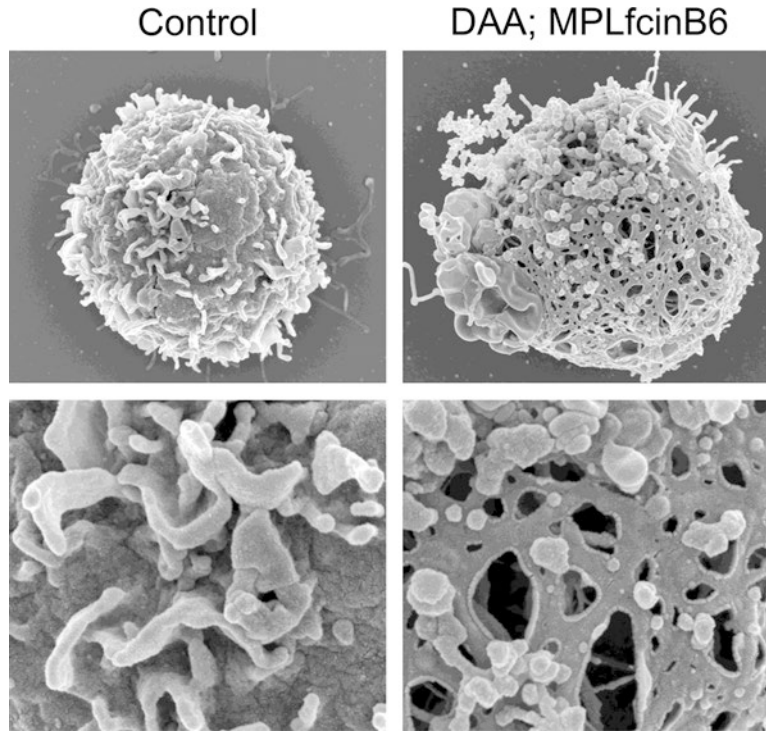

Fig. 9.2 Direct-acting ACPs rapidly lyse human multiple myeloma cells. MPLfcinB6 (50 μM) or its vehicle control were added to U226 human multiple myeloma cells for 2 h. The cells were subsequently fixed, processed, and visualized by scanning electron microscopy. The top and bottom images were captured under 7000 and 40,000× magnification, respectively

Ramamoorthy 2008; Hilchie and Hoskin 2010; Mader and Hoskin 2006; Yeaman and Yount 2003; Bhutia and Maiti 2008; Giuliani et al. 2007).

Owing to the presence of zwitterionic phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin, normal cell membranes are neutral in charge (Zachowski 1993). In contrast, the outer membrane leaflet of cancer cells carries a net negative charge due to increased levels of anionic phosphatidylserine, O-glycosylated mucins, heparan and chondroitin sulfate proteoglycans, and sialylated glycoproteins (Utsugi et al. 1991; Bafna et al. 2010; Koo et al. 2008; van Beek et al. 1973; Iida et al. 1996). Collectively, these differences are thought to contribute to the selective attraction of ACPs to cancer cell membranes. Following the initial stages of peptide binding, ACPs are thought to anchor to the membrane via insertion of hydrophobic residues into the hydrophobic core of the plasma membrane (Hoskin and Ramamoorthy 2008; Hilchie and Hoskin 2010; Mader and Hoskin 2006; Yeaman and Yount 2003; Bhutia and Maiti 2008; Giuliani et al. 2007). Once the peptide is securely bound to the membrane, it either causes membrane instability followed by pore formation and cell lysis (DAAs), or it penetrates into the cytoplasm without substantially damaging the membrane, wherein the peptide initiates apoptosis (indirect-acting ACPs). There are several different models to describe how ACPs cause membrane instability. These models are thoughtfully described elsewhere (Nguyen et al. 2011).

Many studies have used artificial membranes as model systems to show that peptide binding and membrane perturbation are influenced by the lipid content of membranes (Gazit et al. 1995; Matsuzaki et al. 1989). However, it is considerably more difficult to determine the factors that are involved in ACP binding to eukaryotic membranes due to the complexity of the membrane. We demonstrated that the DAAs NRC-03 and NRC-07 exhibit 100- and 50-fold, respectively, greater binding to breast cancer cells than to normal untransformed fibroblasts (Hilchie et al. 2011). In this case, peptide binding was influenced by, but not dependent on, several different anionic surface molecules. Our own work revealed that hundreds of genes are differentially expressed in cancer cells that are refractory to these DAAs (manuscript in preparation). Importantly, these factors appear to influence the toxicity of several DAAs, suggesting a common mechanism of membrane perturbation. Further to this, decreased susceptibility to these DAAs impacted the tumorigenicity of the malignant cells. This work also suggests that dozens of components of the extracellular matrix are likely involved in peptide binding to, and disruption of, the target cell membrane. It is clear that we are only beginning to comprehend the complexity of this process.

9.2.2 Factors That Influence the Mechanism of ACP-Mediated Anticancer Activity

To our knowledge, there is no evidence to suggest that specific structural determinants are responsible for rendering an ACP direct-acting or indirect-acting. Interestingly, in select cases, the mechanism of peptide-mediated cytotoxicity is dependent on the cancer cell line under investigation. For instance, bovine lactoferricin induces apoptosis in human leukemia, lymphoma, and breast cancer cells (Mader et al. 2005; Furlong et al. 2010; Furlong et al. 2006), whereas the same ACP is lytic to fibrosarcoma, melanoma, colorectal cancer, and neuroblastoma cells (Eliassen et al. 2002; Eliassen et al. 2006). In other cases, ACPs may be selectively toxic for one cancer cell type but devoid of effects on another cancer cell type. For example, the ACP MPLfcinB6 selectively lyses leukemia and lymphoma cells (Hilchie et al. 2013b) but is not cytotoxic for breast cancer cells (unpublished). This may be the result of many fundamental differences in the complexity of the membranes of these different types of cancer cells, as we consistently note that cancer cells in suspension (e.g., Jurkat T leukemia cells) are much more susceptible to killing by ACPs than are cancer cells grown as monolayers (e.g., MDA-MB-231 breast carcinoma cells). For instance, the pleurocidins

NRC-03 and NRC-07, as well as the wasp venom peptide mastoparan, are roughly two- to fourfold more toxic to leukemia and myeloma cells than they are to breast carcinoma cells (Hilchie et al. 2011, 2013c, 2016). These findings are further supported by our ongoing quantitative structure/activity relationship studies, which are discussed briefly in Sect. 9.5.2.

As ACPs are small amphipathic molecules with defined secondary structures, it stands to reason that alterations in the amino acid composition of ACPs may affect their potency and mechanism of action. While this has not been studied extensively, we recently noted a striking difference in the mechanism of action of mastoparan by simple C-terminal amidation. Others have shown that unamidated mastoparan kills cancer cells by induction of apoptosis; in contrast, we showed that mastoparan that incorporates a C-terminal amide is much more potent and kills cancer cells by inducing cell lysis (Hilchie et al. 2016; de Azevedo et al. 2015). This finding not only demonstrates the importance of the primary amino acid sequence in determining the mechanism of action of a given ACP but also provides hope that detailed structure/activity relationship studies may reveal next-generation peptides with improved selectivity for cancer cells, thereby addressing one of the most significant limitations to peptide-based therapeutics.

9.3 ACP-Mediated Immune Activation

9.3.1 Cationic Amphipathic Peptides as Modulators of Immune Function

Many cationic peptides were initially characterized for their antimicrobial activities. More recent research shows that many of these same peptides exhibit immune-modulatory activities, as previously reviewed (Hilchie et al. 2013a). Importantly, the antimicrobial activities of many of these peptides are lost in the presence of serum, whereas the immune-modulatory functions of these peptides are maintained under physiologically relevant conditions (Hilchie et al. 2013a).

Some synthetic peptides that have the ability to modulate the immune system are known as innate defense regulators (IDR). Although some of the antibacterial properties of these peptides are lost under physiological conditions, these peptides are still bioactive through immune-modulating effects such as increasing chemokine production (Hilchie et al. 2013a). Monocyte migration in response to chemokines shows a further increase in the presence of the peptide IDR-1002 via a mechanism involving integrins and AKT signaling (Madera and Hancock 2012). IDR-1002 also activates the immune response by increasing chemokine production and by recruiting leukocytes to the site of infection (Nijnik et al. 2010).

Some peptides have the ability to promote antibody production. Immunizing mice with the peptide HH2 along with pertussis toxoid and CpG 10101 significantly increases the titer of toxoid-specific antibodies, indicating that this peptide enhances antibody production in this mouse vaccination model (Kindrachuk et al. 2009).

Despite the many actions of ACPs that promote immune responses, the bioactive peptide lactoferricin B decreases superantigen-mediated interleukin-2 production by mouse splenocytes (Hayworth et al. 2009). This finding indicates that certain ACPs modulate immune function; however, the nature of that modulation may be dependent on the variables present in a given situation.

Mast cells are prominent within tissues and exert multiple effects on the vasculature as a result of their degranulation. Pleurocidins, IDR-1018, and other HDPs induce mast cell degranulation, intracellular calcium mobilization, and the release of prostaglandins (Pundir et al. 2014; Yanashima et al. 2017; Gupta et al. 2016). These peptides may therefore act on mast cells to promote vascular permeability and vasodilation, subsequently shaping the developing immune response.

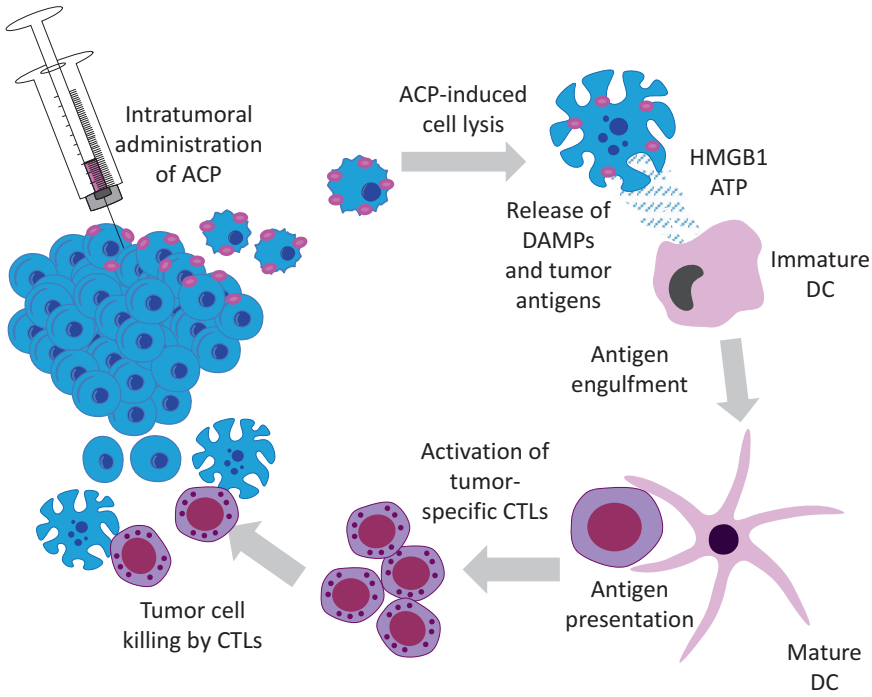

Fig. 9.3 Activation of a protective anti-tumor cytotoxic T lymphocyte (CTL) response by ACPs. In vivo data suggests that a protective immune response develops after intratumoral administration of an ACP. The ACP kills tumor cells, resulting in the release of danger-associated molecular pattern molecules (DAMPs) such as ATP and high mobility group box protein 1 (HMGB1) that promote tumor antigen uptake by dendritic cells (DCs), which then mature and present antigen to T cells. Tumor-specific cytotoxic T lymphocytes (CTLs) are generated that kill tumor cells

9.3.2 Induction of Antitumor Immune Responses by Immunogenic Cell Death

Some ACPs release danger signals from the cell that are thought to be immunogenic. The release of danger-associated molecular patterns (DAMPs) like calreticulin, ATP, and high mobility group box protein 1 (HMGB1) from dying cancer cells results in the induction of an immune response to tumor antigens (Fig. 9.3) (Camilio et al. 2014b).

Intratumoral administration of the bovine lactoferricin-derived ACP LTX-302 to A20 lymphoma-bearing immune-competent mice results in tumor necrosis and inflammatory cell infiltration, followed by complete tumor regression as well as tumor-specific protection against tumor rechallenge. In vitro treatment of these lymphoma cells with LTX-302 results in an increase in HMGB1 release from these cells (Berge et al. 2010). Taken together, these findings suggest that ACP-mediated lysis of malignant cells induces anticancer immunity.

The study of B16 melanoma-bearing mice showed that intratumoral treatment with DAA LTX-315 results in tumor regression and significantly increased survival following tumor rechallenge (Camilio et al. 2014a). In these animals, T cells are recruited to LTX-315-treated tumors, and inflammatory cytokine gene expression is elevated following LTX-315 treatment. Mice that were previously cured of palpable melanoma with LTX-315 treatment are protected from rechallenge with B16 melanoma cells (Camilio et al. 2014a). In vitro LTX-315 treatment of melanoma cells releases DAMPs that include ATP, cytochrome C, reactive oxygen species (ROS), and HMGB1 (Camilio et al. 2014a; Eike et al. 2015).

ACPs that induce a local immune response in tumors may also trigger a systemic immune response that removes all neoplastic cells, including those that have spread to other parts of the body. This immune response is activated by the ACP-induced release of DAMPs. Inhibition of local regulatory T cells (Tregs) at the tumor site is another aspect to consider since inhibiting these cellular regulators of the immune response is known to promote anticancer immune responses. In tumor beds, LTX-315 increased the number of $CD4^+$ (Th1 and Th17) and $CD8^+$ T cells while decreasing Treg numbers (Yamazaki et al. 2016). The cationic peptide LL-37, which has both pro- and anticancer effects depending on the cancer (Chen et al. 2018), also inhibits $CD25^+CD4^+FOXP3^+$ T regulatory cells and so may be helpful in promoting an anticancer immune response (Mader et al. 2011).

Administration of LTX-315 increased CTLA-4 expression on $CD8^+$ T cells while decreasing PD-1 expression, suggesting that using this ACP in combination with an inhibitor of CTLA-4 (ipilimumab) may improve treatment outcome (Yamazaki et al. 2016). Initial experiments with immune checkpoint inhibitors such as ipilimumab suggest that timing of the treatments may be critical as administration of the CTLA-4 neutralizing agent prior to the treatment with the ACP, LTX-315, may be needed to achieve a therapeutic benefit. The need for treatment with ipilimumab in advance of ACP administration is explained by the fact that CTLA-4 is involved in down-regulating T-cell activation.

9.3.3 Comparison of ACPs to Oncolytic Virus Therapy

Oncolytic viruses are another class of novel therapeutics being investigated for the management of various cancers. Oncolytic viruses may fail to kill tumor cells in an individual if the virus is quickly eliminated as the result of triggering an innate immune response (Chiocca and Rabkin 2014). The first oncolytic virus derived from a genetically modified type 1 herpes simplex virus (HSV) has been approved for use and shows therapeutic benefit to melanoma patients (Andtbacka et al. 2015). However, only a moderate increase in survival is reported with this oncolytic virus therapy, indicating other treatments are needed. Another oncolytic virus, vaccinia JX-594, has been used to treat liver cancer patients, in which the virus was shown to be oncolytic and increase patient survival with some evidence of an immune-activating mechanism (Heo et al. 2013). Therefore, evidence exists that treatment with certain oncolytic viruses is able to increase the survival of cancer patients.

Oncolytic peptides, as a result of their short half-life, may provide a safer alternative for patients in comparison to oncolytic viruses. There are safety concerns when patients are administered a virus that may persist long term and has the potential to mutate into a harmful variant. Some ACPs are active against drug-resistant cancer cells and are not lytic for red blood cells, making them potential candidates for development as future treatments for cancer. Since some ACPs kill cancer cells and induce an anticancer immune response (Haug et al. 2016), injection of LTX-315 into transdermally accessible tumors is currently in phase I clinical trials to assess safety, dosing, pharmacokinetics, and immune response development (ClinicalTrials.gov (NCT01058616) 2010). LTX-315 is also now being assessed in multiple cancers as a monotherapy or in combination with ipilimumab or pembrolizumab (ClinicalTrials.gov (NCT01986426) 2013). Discovery research has identified these oncolytic peptides and has revealed their in vitro and in vivo activities. These ACPs are now being examined for clinical efficacy. Even in phase I clinical trials, there is assessment of ACP antitumor activity as indicated by complete and partial response rates, overall response rate, and progression-free survival. The results of these trials will begin to answer questions regarding the effectiveness of ACPs and the potential benefit of enhanced delivery of these oncolytic peptides.

9.4 Strategies to Enhance ACP Delivery

Since peptides typically undergo rapid degradation in the body, a delivery platform may be needed to ensure that ACPs get to their desired target. This may not be necessary for fast-acting peptides; nevertheless, methods to package ACPs so that they reach the tumor microenvironment include the use of nanoparticles and fusogenic liposomes. Peptide modification strategies can also be used to promote tumor cell targeting; however, this approach will be discussed in Sect. 9.5.

9.4.1 Nanoparticles

Nanoparticles provide a mechanism for drug delivery to the correct location in patients, including those with drug-resistant cancers. Many different nanoparticle formulations have been considered. The nanoparticles themselves need to be stable and nontoxic, and they must be targetable in order to deliver the drug of interest to the correct cell/tissue.

Perfluorocarbon nanoparticles have been of particular interest for drug delivery because these nanoparticles are biologically inert, stable, nontoxic, and can be monitored using different imaging platforms. Perfluorocarbon nanoparticles can carry large quantities of drugs, and their delivery to target sites can be observed in vivo (Winter 2014; Chen et al. 2013). Since ACPs are small, it is feasible to put ACPs on/in these perfluorocarbon nanoparticles to enhance their delivery to a primary tumor and metastatic lesions. Studies that used perfluorocarbon nanoparticles loaded with melittin, a cytolytic peptide from bee venom, have revealed that the combination of an ACP and nanoparticle delivery system is able to significantly decrease B16 melanoma tumor volumes in vivo (Soman et al. 2009; Pan et al. 2011).

Since the microenvironment of many solid tumors is acidic (Tannock and Rotin 1989), some nanoparticle delivery approaches have been engineered to function best at low pH. For example, a CPMSN nanocarrier bearing the arginine-glycine-aspartic acid (RGD) peptide on its surface is taken up by breast cancer cells via integrin receptor-mediated endocytosis and has been engineered to subsequently degrade in the acidic endosomal compartment, resulting in the intracellular release of cytotoxic anticancer drugs (Murugan et al. 2016). It should be possible to use a similar approach to deliver ACPs directly into the acidic tumor microenvironment.

Some nanocarriers are toxic on their own, especially those made with polyacrylic acids, indicating the need for nontoxic nanocarriers. In this regard, doxorubicin has been encapsulated within a nanocarrier made of 30% oxidized starch and decorated with an integrin-targeting peptide attached with a polyethylene glycol (PEG) linker in order to selectively target integrin-overexpressing cancer cells (Jiang et al. 2018). Such a starch-based nanocarrier is likely to be less toxic than other nanoparticle formulations.

Another strategy for delivering ACPs to cancer cells is through the use of fusogenic liposomes, which are able to deliver hydrophobic or hydrophilic drugs directly into a target cell without risking degradation by the endocytic pathway (Kube et al. 2017). Fusogenic liposomes that effect membrane fusion have been used to deliver LfcinB6 into the cytoplasmic compartment of both leukemia cells and breast cancer cells, resulting in rapid cytotoxicity (Richardson et al. 2009). In addition to the potential for tumor cell targeting, fusogenic liposomes are expected to protect ACPs from proteolysis long enough for them to reach effective concentrations in the tumor site.

Clearly, there are multiple strategies that can be employed to effectively deliver ACPs to cancer cells. The next critical step will be to evaluate the safest approach in phase I clinical trials. Indeed, multiple clinical trials in which nanoparticles are being used to deliver different chemotherapeutic agents (e.g., paclitaxel) are underway. Since there are already ongoing clinical trials with ACPs, such as LTX-315, future use of nanoparticles as delivery vehicles for ACPs may be an effective strategy to increase the half-life of oncolytic peptides.

9.4.2 Peptides with Altered Stereochemistry

One potential problem with ACP-based treatments is that these peptides can be easily degraded by proteolytic enzymes present in the digestive system and blood plasma (Vlieghe et al. 2010). Susceptibility to degradation is dependent on the peptide sequence (e.g., trypsin cleaves arginine and lysine); however, altering the stereochemistry of an ACP may render it unrecognizable by proteolytic enzymes. In this regard, since amino acids occur naturally as an "L" stereoisomer, D-isomers are not susceptible to proteolytic degradation (Hilchie et al. 2015). For example, an all-D-amino acid variant of pleurocidin that is based on the L form of the cationic antimicrobial peptide from winter flounder resists degradation by trypsin, plasmin, and carboxypeptidase (Jung et al. 2007). Findings such as this indicate that the stereochemistry of a peptide is relevant with respect to its susceptibility to degradation.

9.4.3 Potential for ACP-Expressing Oncolytic Virus Therapy

If clinical trials continue to show that oncolytic viruses are safe and effective anticancer agents, it may be advantageous to engineer an oncolytic virus that also expresses a direct- or indirect-acting ACP. Since the mechanism of oncolysis is different between oncolytic viruses and ACPs, the potential exists for an enhanced cytotoxic effect by an ACP-expressing oncolytic virus. Administration of an oncolytic virus that also codes for an oncolytic peptide is predicted to increase the likelihood of killing all cancer cells in a given tissue, including cancer stem cells, and activating a long-lasting anticancer immune response that will protect against cancer recurrence.

9.5 Strategies to Enhance ACP Selectivity for Cancer Cells

ACPs, particularly those that are direct-acting, have many advantages over conventional chemotherapeutic agents; however, ACPs continue to be limited by their toxicity to normal human cells at *high* peptide concentrations. Several strategies have been used to improve ACP selectivity for cancer cells. Many of these strategies involve optimizing the delivery of peptide to tumor cells through the use of nanoparticle-based delivery systems, as discussed in Sect. 9.4.1. Here, we will briefly review how alterations in the primary amino acid sequence influence the selectivity of ACPs for cancer cells.

9.5.1 Generating Tumor-Specific ACPs Through the Addition of Peptide-Targeting Motifs

As noted in Sect. 9.1.4, ACP selectivity for cancer cells can be enhanced through the addition of so-called targeting sequences. This strategy involves the use of a glycine-glycine linker to conjugate the ACP with a peptide sequence that recognizes specific molecules that are overexpressed by cancer cells. The targeting motif then promotes ACP binding to the tumor cell, after which the cytotoxic portion of the peptide triggers cell death. There are dozens of examples of targeting sequences and many instances in which this strategy has been used to improve ACP selectivity – some have been successful, whereas others have not enjoyed success. Here, we will provide an example of each strategy for the purpose of illustration.

Bombesin is a 14-residue tumor-homing peptide that binds several receptors that are overexpressed by many cancer cell types (Anastasi et al. 1971; Reubi et al. 2002; Cornelio et al. 2007). Significant improvements in tumor cell killing were noted when magainin 2 was conjugated to bombesin (Liu et al. 2011). In comparison to the parental peptide (magainin 2), the IC_{50} of the hybrid peptide for cancer cells was at least tenfold lower, which was substantially lower than the IC_{50} for normal cells. This finding suggests that the increase in potency of the hybrid peptide was not at the expense of cancer cell selectivity.

Phage display libraries can be used to identify novel targeting sequences for ACPs. For example, a screen of phage display libraries was used

to identify the sequence LTVSPWY, which has been successfully used to deliver oligonucleotides to SKBR3 breast cancer cells (Shadidi and Sioud 2003). However, this sequence did not improve the cytotoxicity of LfcinB6 for a different breast cancer cell line (unpublished), indicating the need to screen for broad applicability of targeting sequences in a particular type of cancer.

9.5.2 Enhancing ACP Selectivity Through Amino Acid Substitution/Modification

It is no secret that slight alterations to the primary amino acid sequence can drastically affect the potency of an ACP. In some cases, a minor alteration may even change the mechanism of action of the peptide. For example, the addition of a C-terminal amide causes the wasp venom peptide mastoparan to become lytic, whereas in its unamidated form, mastoparan induces mitochondria-dependent apoptosis (Hilchie et al. 2016; de Azevedo et al. 2015).

Many groups have attempted to improve peptide selectivity through amino acid substitution. The vast majority of these studies use hypothesis-driven, small-scale approaches, whereby charge and/or hydrophobicity of the parent ACP is modified, based on the knowledge that these features are required for cancer cell killing (Hoskin and Ramamoorthy 2008). Such studies generate a very small peptide library that is subsequently screened for cytotoxic activity against cancer cells and normal cells. This approach has been used to identify novel peptides with slightly increased selectivity for cancer cells (Dennison et al. 2006; Yang et al. 2003; Eliassen et al. 2003; Arias et al. 2017). Often, many incremental improvements are needed before one obtains an ACP with significantly improved selectivity relative to the parent peptide, likely because we still do not understand how the overall structure of the ACP affects its selectivity for cancer cells. To our knowledge, only one study has examined the effect of altered charge and hydrophobicity on cancer cell selectivity in the context of the overall structure of the ACP (Yang et al. 2002). In this study, helical wheel diagrams of the parent peptide were used to show that positively charged amino acids cluster into two spatially separated regions, termed the major and minor sector, that contain four and two cationic amino acids, respectively. Moving the two cationic amino acids from the minor sector to the major sector increased cancer cell killing at the expense of cancer cell selectivity, suggesting that the presence of a minor sector may reduce ACP toxicity to normal cells. The authors also noted that increasing the overall charge of the ACP by the addition of two additional cationic amino acids to the major sector resulted in reduced potency; however, selectivity for malignant cells was maintained, most likely because the addition of these two amino acids occurred at the expense of two hydrophobic amino acids.

In spite of numerous efforts to generate next-generation ACPs with improved selectivity for cancer cells, we still do not really understand the structural basis for cancer cell selectivity. It is our opinion that this is due to the lack of available datasets that are sufficiently large to conduct thorough structure/activity relationship (SAR) studies. To this end, we have used SPOT array technologies to create a massive peptide library ($n = 210$), which we then screened for cytotoxic activity against cancer cells and normal cells (manuscript in preparation). We found that single amino acid substitutions may eliminate cytotoxicity for both cancer cells and normal cells, eliminate selectivity for cancer cells, and/or improve selectivity for leukemia and/or breast cancer cells. Our goal is to use an artificial intelligence approach to predict highly selective next-generation ACPs through computer modeling of quantitative structure/activity relationships (QSAR), which yielded hundreds of peptides predicted to be more selective for cancer cells than the parent ACP. Efforts to screen this new peptide library are underway. While this study is in its infancy, we are confident that highly selective ACPs will be identified as this approach has successfully delivered novel peptides with improved antimicrobial and anti-biofilm activities (Hilpert et al. 2005; Haney et al. 2018).

9.5.3 Improving Tumor Selectivity Through Histidine Substitution

It is well established that the microenvironment of solid tumors is acidic in comparison to most normal tissues due to lactic acid buildup coupled with inadequate washout of acidic products as a result of inadequate vascularization (Tannock and Rotin 1989; Newell et al. 1993; Vaupel et al. 1989). Yechiel Shai's group has used these differences in tumor microenvironment to optimize ACP selectivity through the use of histidine substitutions (Makovitzki et al. 2009). In this innovative approach, three or six lysine residues in the ACP [D]-K_6L_9 (pKa ~10.5) were replaced with histidine residues (pKa ~6.1), generating [D]-$K_3H_3L_9$ and [D]-H_6L_9, respectively. Unlike [D]-K_6L_9, neither [D]-$K_3H_3L_9$ nor [D]-H_6L_9 had adverse toxic side effects when delivered to mice via intravenous injection, and both ACPs caused a reduction in the growth of prostate tumor xenografts in mice. These results provide the intriguing possibility of customizing peptides for selective targeting of the solid tumor microenvironment, thereby sparing healthy tissues from potential adverse side effects. Despite these exciting results, to our knowledge, this proof of concept work has not been replicated with other ACPs. However, it is worth noting that our QSAR analysis predicts that peptide selectivity for breast carcinoma cells often involves histidine substitutions (manuscript in preparation).

9.6 The Future of ACP Research

Cancer cells are increasingly resistant to conventional treatment modalities. As patient survival increases, so does the risk of recurrent disease in a form that is resistant to previously used drugs. Peptide-based therapies have the potential to treat many different cancers, including those that are multidrug resistant or slow growing and therefore not susceptible to conventional chemotherapy. Unfortunately, research in this area has been slow to progress. The anticancer potential of magainins has been appreciated since at least the 1980s without any significant progress to clinical trials. Areas in which further study is essential include ascertaining the immuno-modulatory properties of ACPs and improving their selectivity for cancer cells under physiologically relevant conditions. Indeed, ACP-mediated induction of danger signals and the subsequent development of anticancer immune responses may be essential for long-term benefit and increased patient survival. Moreover, improved selectivity is essential for future clinical trials to ensure the safety and efficacy of ACP administration to cancer patients. ACPs must be able to kill cancer cells without adverse toxicities. Although it is possible that a single ACP may be effective against all cancer types, it is more likely that different ACPs will be needed to treat different cancers. Computer modeling may help to advance this area of research so that future treatments can be identified and assessed at a more rapid pace. In addition, computer models may predict highly selective ACPs that also activate antitumor immune responses.

Treating patients with combinational therapies including novel drugs like ACPs is going to be essential to combat multidrug-resistant cancers. Generation of ACP-producing oncolytic viruses may be an additional area for future study to combat recurrence and multidrug-resistant cancers. Finally, the use of tumoricidal ACPs in combination with conventional cytotoxic drugs is likely to improve patient survival by more effective lysis of tumor cells with reduced treatment-related toxicities and a reduction in the risk of tumor recurrence as the result of the generation of long-lasting tumor-specific immunity.

References

Anastasi A, Erspamer V, Bucci M (1971) Isolation and structure of bombesin and alytesin, 2 analogous active peptides from the skin of the European amphibians Bombina and Alytes. Experientia 27:166–167

Andtbacka RHI et al (2015) Talimogene Laherparepvec improves durable response rate in patients with advanced melanoma. J Clin Oncol 33:2780–2788

Arias M et al (2017) Anticancer activities of bovine and human lactoferricin-derived peptides. Biochem Cell Biol 95:91–98

Bafna S, Kaur S, Batra SK (2010) Membrane-bound mucins: the mechanistic basis for alterations in the growth and survival of cancer cells. Oncogene 29:2893–2904

Baker MA, Maloy WL, Zasloff M, Jacob LS (1993) Anticancer efficacy of magainin2 and analogue peptides. Cancer Res 53:3052–3057

Berge G et al (2010) Therapeutic vaccination against a murine lymphoma by intratumoral injection of a cationic anticancer peptide. Cancer Immunol Immunother 59:1285–1294

Bergman P, Walter-Jallow L, Broliden K, Agerberth B, Söderlund J (2007) The antimicrobial peptide LL-37 inhibits HIV-1 replication. Curr HIV Res 5:410–415

Bhutia SK, Maiti TK (2008) Targeting tumors with peptides from natural sources. Trends Biotechnol 26:210–217

Bush JA, Li G (2002) Cancer chemoresistance: the relationship between p53 and multidrug transporters. Int J Cancer 98:323–330

Camilio KA, Berge G, Ravuri CS, Rekdal O, Sveinbjørnsson B (2014a) Complete regression and systemic protective immune responses obtained in B16 melanomas after treatment with LTX-315. Cancer Immunol Immunother 63:601–613

Camilio KA, Rekdal O, Sveinbjörnsson B (2014b) LTX-315 (Oncopore™): a short synthetic anticancer peptide and novel immunotherapeutic agent. Oncoimmunology 3:e29181

Chen J, Pan H, Lanza GM, Wickline SA (2013) Perfluorocarbon nanoparticles for physiological and molecular imaging and therapy. Adv Chronic Kidney Dis 20:466–478

Chen X et al (2018) Roles and mechanisms of human cathelicidin LL-37 in cancer. Cell Physiol Biochem 47:1060–1073

Chiocca EA, Rabkin SD (2014) Oncolytic viruses and their application to cancer immunotherapy. Cancer Immunol Res 2:295–300

Choi DK, Helenowski I, Hijiya N (2014) Secondary malignancies in pediatric cancer survivors: perspectives and review of the literature. Int J Cancer 135:1764–1773

ClinicalTrials.gov (NCT01058616) (2010)

ClinicalTrials.gov (NCT01986426) (2013)

Cornelio DB, Roesler R, Schwartsmann G (2007) Gastrin-releasing peptide receptor as a molecular target in experimental anticancer therapy. Ann Oncol 18:1457–1466

Couto J et al (2018) Antiplasmodial activity of tick defensins in a mouse model of malaria. Ticks Tick-Borne Dis 9:844–849

de Azevedo RA et al (2015) Mastoparan induces apoptosis in B16F10-Nex2 melanoma cells via the intrinsic mitochondrial pathway and displays antitumor activity in vivo. Peptides 68:113–119

de la Fuente-Núñez C et al (2012) Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide. Antimicrob Agents Chemother 56:2696–2704

Dennison SR, Whittaker M, Harris F, Phoenix DA (2006) Anticancer alpha-helical peptides and structure/function relationships underpinning their interactions with tumour cell membranes. Curr Protein Pept Sci 7:487–499

Donnelly JG (2004) Pharmacogenetics in cancer chemotherapy: balancing toxicity and response. Ther Drug Monit 26:231–235

Eike L-M, Yang N, Rekdal Ø, Sveinbjørnsson B (2015) The oncolytic peptide LTX-315 induces cell death and DAMP release by mitochondria distortion in human melanoma cells. Oncotarget 6:34910–34923

Eliassen LT et al (2002) Evidence for a direct antitumor mechanism of action of bovine lactoferricin. Anticancer Res 22:2703–2710

Eliassen LT, Haug BE, Berge G, Rekdal O (2003) Enhanced antitumour activity of 15-residue bovine lactoferricin derivatives containing bulky aromatic amino acids and lipophilic N-terminal modifications. J Pept Sci 9:510–517

Eliassen LT et al (2006) The antimicrobial peptide, lactoferricin B, is cytotoxic to neuroblastoma cells in vitro and inhibits xenograft growth in vivo. Int J Cancer 119:493–500

Furlong SJ, Mader JS, Hoskin DW (2006) Lactoferricin-induced apoptosis in estrogen-nonresponsive MDA-MB-435 breast cancer cells is enhanced by C6 ceramide or tamoxifen. Oncol Rep 15:1385–1390

Furlong SJ, Mader JS, Hoskin DW (2010) Bovine lactoferricin induces caspase-independent apoptosis in human B-lymphoma cells and extends the survival of immune-deficient mice bearing B-lymphoma xenografts. Exp Mol Pathol 88:371–375

Furue M et al (2018) Melanoma and immune checkpoint inhibitors. Curr Oncol Rep 20:29

Gazit E, Boman A, Boman HG, Shai Y (1995) Interaction of the mammalian antibacterial peptide cecropin P1 with phospholipid vesicles. Biochemistry (Mosc) 34:11479–11488

Giuliani A, Pirri G, Nicoletto S (2007) Antimicrobial peptides: an overview of a promising class of therapeutics. Cent Eur J Biol 2:1–33

Greenshields AL, Knickle LC, Syvitski R, Douglas SE (2008) Strategies for recombinant expression of small, highly disulphide-bonded, cationic antimicrobial peptides. Protein Pept Lett 15:985–994

Gupta K, Subramanian H, Ali H (2016) Modulation of host defense peptide-mediated human mast cell activation by LPS. Innate Immun 22:21–30

Haney EF et al (2018) Computer-aided discovery of peptides that specifically attack bacterial biofilms. Sci Rep 8:1871

Hansel W, Enright F, Leuschner C (2007) Destruction of breast cancers and their metastases by lytic peptide conjugates in vitro and in vivo. Mol Cell Endocrinol 260–262:183–189

Haug BE et al (2016) Discovery of a 9-mer cationic peptide (LTX-315) as a potential first in class oncolytic peptide. J Med Chem 59:2918–2927

Hayworth JL et al (2009) Attenuation of massive cytokine response to the staphylococcal enterotoxin B superantigen by the innate immunomodulatory protein lactoferrin. Clin Exp Immunol 157:60–70

Heo J et al (2013) Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. Nat Med 19:329–336

Hilchie AL, Hoskin DW (2010) Application of cationic antimicrobial peptides in cancer treatment: laboratory investigations and clinical potential. In: Emerging cancer therapy: microbial approaches and biotechnological tools. Wiley, Hoboken, pp 309–332

Hilchie AL et al (2011) Pleurocidin-family cationic antimicrobial peptides are cytolytic for breast carcinoma cells and prevent growth of tumor xenografts. Breast Cancer Res 13:R102

Hilchie AL, Power Coombs MR, Hoskin DW (2012) Obstacles and solutions to the use of cationic antimicrobial peptides in the treatment of cancer. In: Small wonders: peptides for disease control. American Chemical Society/Oxford University Press, Washington, DC/Oxford, pp 61–78

Hilchie AL, Wuerth K, Hancock REW (2013a) Immune modulation by multifaceted cationic host defense (antimicrobial) peptides. Nat Chem Biol 9:761–768

Hilchie AL, Vale R, Zemlak TS, Hoskin DW (2013b) Generation of a hematologic malignancy-selective membranolytic peptide from the antimicrobial core (RRWQWR) of bovine lactoferricin. Exp Mol Pathol 95:192–198

Hilchie AL et al (2013c) Pleurocidin-family cationic antimicrobial peptides mediate lysis of multiple myeloma cells and impair the growth of multiple myeloma xenografts. Leuk Lymphoma 54:2255–2262

Hilchie AL, Haney EF, Pinto DM, Hancock REW, Hoskin DW (2015) Enhanced killing of breast cancer cells by a d-amino acid analog of the winter flounder-derived pleurocidin NRC-03. Exp Mol Pathol 99:426–434

Hilchie AL et al (2016) Mastoparan is a membranolytic anti-cancer peptide that works synergistically with gemcitabine in a mouse model of mammary carcinoma. Biochim Biophys Acta 1858:3195–3204

Hilpert K, Volkmer-Engert R, Walter T, Hancock REW (2005) High-throughput generation of small antibacterial peptides with improved activity. Nat Biotechnol 23:1008–1012

Hoskin DW, Ramamoorthy A (2008) Studies on anticancer activities of antimicrobial peptides. Biochim Biophys Acta 1778:357–375

Hui L, Leung K, Chen HM (2002) The combined effects of antibacterial peptide cecropin A and anti-cancer agents on leukemia cells. Anticancer Res 22:2811–2816

Iida J, Meijne AM, Knutson JR, Furcht LT, McCarthy JB (1996) Cell surface chondroitin sulfate proteoglycans in tumor cell adhesion, motility and invasion. Semin Cancer Biol 7:155–162

Ishida H, Nguyen LT, Gopal R, Aizawa T, Vogel HJ (2016) Overexpression of antimicrobial, anticancer, and transmembrane peptides in *Escherichia coli* through a calmodulin-peptide fusion system. J Am Chem Soc 138:11318–11326

Jean F, Tomasini P, Barlesi F (2017) Atezolizumab: feasible second-line therapy for patients with non-small cell lung cancer? A review of efficacy, safety and place in therapy. Ther Adv Med Oncol 9:769–779

Jiang Y et al (2018) TEMPO-oxidized starch nanoassemblies of negligible toxicity compared with polyacrylic acids for high performance anti-cancer therapy. Int J Pharm 547:520–529

Johnstone SA, Gelmon K, Mayer LD, Hancock RE, Bally MB (2000) In vitro characterization of the anticancer activity of membrane-active cationic peptides. I. Peptide-mediated cytotoxicity and peptide-enhanced cytotoxic activity of doxorubicin against wild-type and p-glycoprotein over-expressing tumor cell lines. Anticancer Drug Des 15:151–160

Jung HJ et al (2007) Fungicidal effect of pleurocidin by membrane-active mechanism and design of enantiomeric analogue for proteolytic resistance. Biochim Biophys Acta 1768:1400–1405

Kebudi R, Ozdemir GN (2017) Secondary neoplasms in children treated for cancer. Curr Pediatr Rev 13:34–41

Kim S, Kim SS, Bang YJ, Kim SJ, Lee BJ (2003) In vitro activities of native and designed peptide antibiotics against drug sensitive and resistant tumor cell lines. Peptides 24:945–953

Kindrachuk J et al (2009) A novel vaccine adjuvant comprised of a synthetic innate defence regulator peptide and CpG oligonucleotide links innate and adaptive immunity. Vaccine 27:4662–4671

Koo C-Y, Sen Y-P, Bay B-H, Yip GW (2008) Targeting heparan sulfate proteoglycans in breast cancer treatment. Recent Pat Anticancer Drug Discov 3:151–158

Kube S et al (2017) Fusogenic liposomes as nanocarriers for the delivery of intracellular proteins. Langmuir 33:1051–1059

Leuschner C, Hansel W (2004) Membrane disrupting lytic peptides for cancer treatments. Curr Pharm Des 10:2299–2310

Liu S et al (2011) Enhancement of cytotoxicity of antimicrobial peptide magainin II in tumor cells by bombesin-targeted delivery. Acta Pharmacol Sin 32:79–88

Mader JS, Hoskin DW (2006) Cationic antimicrobial peptides as novel cytotoxic agents for cancer treatment. Expert Opin Investig Drugs 15:933–946

Mader JS, Salsman J, Conrad DM, Hoskin DW (2005) Bovine lactoferricin selectively induces apoptosis in human leukemia and carcinoma cell lines. Mol Cancer Ther 4:612–624

Mader JS, Ewen C, Hancock REW, Bleackley RC (2011) The human cathelicidin, LL-37, induces granzyme-mediated apoptosis in regulatory T cells. J Immunother 34:229–235

Madera L, Hancock REW (2012) Synthetic immunomodulatory peptide IDR-1002 enhances monocyte migration and adhesion on fibronectin. J Innate Immun 4:553–568

Makovitzki A, Fink A, Shai Y (2009) Suppression of human solid tumor growth in mice by intratumor and systemic inoculation of histidine-rich and pH-dependent host defense-like lytic peptides. Cancer Res 69:3458–3463

Matsuzaki K et al (1989) Magainin 1-induced leakage of entrapped calcein out of negatively-charged lipid vesicles. Biochim Biophys Acta 981:130–134

Murugan C et al (2016) Combinatorial nanocarrier based drug delivery approach for amalgamation of anti-tumor agents in breast cancer cells: an improved nano-medicine strategies. Sci Rep 6:34053

Nagy P et al (2005) Decreased accessibility and lack of activation of ErbB2 in JIMT-1, a herceptin-resistant, MUC4-expressing breast cancer cell line. Cancer Res 65:473–482

Naumov GN et al (2003) Ineffectiveness of doxorubicin treatment on solitary dormant mammary carcinoma cells or late-developing metastases. Breast Cancer Res Treat 82:199–206

Newell K, Franchi A, Pouysségur J, Tannock I (1993) Studies with glycolysis-deficient cells suggest that production of lactic acid is not the only cause of tumor acidity. Proc Natl Acad Sci U S A 90:1127–1131

Nguyen LT, Haney EF, Vogel HJ (2011) The expanding scope of antimicrobial peptide structures and their modes of action. Trends Biotechnol 29:464–472

Nijnik A et al (2010) Synthetic cationic peptide IDR-1002 provides protection against bacterial infections through chemokine induction and enhanced leukocyte recruitment. J Immunol 184:2539–2550

Overhage J et al (2008) Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect Immun 76:4176–4182

Pan H, Soman NR, Schlesinger PH, Lanza GM, Wickline SA (2011) Cytolytic peptide nanoparticles ('NanoBees') for cancer therapy. Wiley Interdiscip Rev Nanomed Nanobiotechnol 3:318–327

Pillai RN et al (2018) Comparison of the toxicity profile of PD-1 versus PD-L1 inhibitors in non-small cell lung cancer: a systematic analysis of the literature. Cancer 124:271–277

Pundir P, Catalli A, Leggiadro C, Douglas SE, Kulka M (2014) Pleurocidin, a novel antimicrobial peptide, induces human mast cell activation through the FPRL1 receptor. Mucosal Immunol 7:177–187

Reubi JC, Wenger S, Schmuckli-Maurer J, Schaer J-C, Gugger M (2002) Bombesin receptor subtypes in human cancers: detection with the universal radioligand ^{125}I-[D-TYR(6), β-ALA(11), PHE(13), NLE(14)] bombesin(6-14). Clin Cancer Res 8:1139–1146

Richardson A, de Antueno R, Duncan R, Hoskin DW (2009) Intracellular delivery of bovine lactoferricin's antimicrobial core (RRWQWR) kills T-leukemia cells. Biochem Biophys Res Commun 388:736–741

Seidel JA, Otsuka A, Kabashima K (2018) Anti-PD-1 and anti-CTLA-4 therapies in cancer: mechanisms of action, efficacy, and limitations. Front Oncol 8:86

Shadidi M, Sioud M (2003) Identification of novel carrier peptides for the specific delivery of therapeutics into cancer cells. FASEB J 17:256–258

Sheu MJ, Baldwin WW, Brunson KW (1985) Cytotoxicity of rabbit macrophage peptides MCP-1 and MCP-2 for mouse tumor cells. Antimicrob Agents Chemother 28:626–629

Soman NR et al (2009) Molecularly targeted nanocarriers deliver the cytolytic peptide melittin specifically to tumor cells in mice, reducing tumor growth. J Clin Invest 119:2830–2842

Steinstraesser L et al (2012) Innate defense regulator peptide 1018 in wound healing and wound infection. PLoS One 7:e39373

Tannock IF, Rotin D (1989) Acid pH in tumors and its potential for therapeutic exploitation. Cancer Res 49:4373–4384

Thirman MJ, Larson RA (1996) Therapy-related myeloid leukemia. Hematol Oncol Clin North Am 10:293–320

Utsugi T, Schroit AJ, Connor J, Bucana CD, Fidler IJ (1991) Elevated expression of phosphatidylserine in the outer membrane leaflet of human tumor cells and recognition by activated human blood monocytes. Cancer Res 51:3062–3066

van Beek WP, Smets LA, Emmelot P (1973) Increased sialic acid density in surface glycoprotein of transformed and malignant cells – a general phenomenon? Cancer Res 33:2913–2922

Vaupel P, Kallinowski F, Okunieff P (1989) Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. Cancer Res 49:6449–6465

Vlieghe P, Lisowski V, Martinez J, Khrestchatisky M (2010) Synthetic therapeutic peptides: science and market. Drug Discov Today 15:40–56

Wang G, Watson KM, Buckheit RW (2008) Anti-human immunodeficiency virus type 1 activities of antimicrobial peptides derived from human and bovine cathelicidins. Antimicrob Agents Chemother 52:3438–3440

Winter PM (2014) Perfluorocarbon nanoparticles: evolution of a multimodality and multifunctional imaging agent. Scientifica 2014:746574

Yamazaki T et al (2016) The oncolytic peptide LTX-315 overcomes resistance of cancers to immunotherapy with CTLA4 checkpoint blockade. Cell Death Differ 23:1004–1015

Yanashima K et al (2017) Innate defense regulator IDR-1018 activates human mast cells through G protein-, phospholipase C-, MAPK- and NF-κB-sensitive pathways. Immunol Res 65:920–931

Yang N, Stensen W, Svendsen JS, Rekdal Ø (2002) Enhanced antitumor activity and selectivity of lactoferrin-derived peptides. J Pept Res 60:187–197

Yang N, Lejon T, Rekdal O (2003) Antitumour activity and specificity as a function of substitutions in the lipophilic sector of helical lactoferrin-derived peptide. J Pept Sci 9:300–311

Yeaman MR, Yount NY (2003) Mechanisms of antimicrobial peptide action and resistance. Pharmacol Rev 55:27–55

Zachowski A (1993) Phospholipids in animal eukaryotic membranes: transverse asymmetry and movement. Biochem J 294(Pt 1):1–14

Zitzmann S, Ehemann V, Schwab M (2002) Arginine-glycine-aspartic acid (RGD)-peptide binds to both tumor and tumor-endothelial cells in vivo. Cancer Res 62:5139–5143