CMLS, Cell. Mol. Life Sci. 62 (2005) 784–790
1420-682X/05/080784-07
DOI 10.1007/s00018-005-4560-2

CMLS Cellular and Molecular Life Sciences

Visions & Reflections

Host defense peptides as new weapons in cancer treatment

N. Papo and Y. Shai*

Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot 76100 (Israel),
Fax: +972 8 9344112, e-mail: Yechiel.Shai@ weizmann.ac.il

Received 21 December 2004; received after revision 28 January 2005; accepted 1 February 2005

Abstract. In the last decade intensive research has been conducted to determine the role of innate immunity host defense peptides (also termed antimicrobial peptides) in the killing of prokaryotic and eukaryotic cells. Many antimicrobial peptides damage the cellular membrane as part of their killing mechanism. However, it is not clear what makes cancer cells more susceptible to some of these peptides, and what the molecular mechanisms underlying these activities are. Two general mechanisms were suggested: (i) plasma membrane disruption via micellization or pore formation, and (ii) induction of apoptosis via mitochondrial membrane disruption. To be clinically used, these peptides need to combine high and specific anticancer activity with stability in serum. Although so far very limited, new studies have paved the way for promising anticancer host defense peptides with a new mode of action and with a broad spectrum of anticancer activity.

Key words. Host defense peptides; cytolytic peptides; lytic peptides; anticancer peptides; amphipatic peptides; model membranes; antimicrobial peptides; membrane permeabilization.

Introduction

Current anticancer chemotherapies that are based on alkylating agents, antimetabolites and natural products are heterogeneous in their mode of action, and most of them also act against normal mammalian cells, consequently causing severe side effects [1]. In addition, these compounds need to penetrate into the target cell in order to function. As a consequence, the cell can develop resistance by pumping the drugs out using multi-drug-resistant proteins [2].

The human body uses its immune system in order to recognize and destroy clones of cancer cells [3, 4]. Despite evidence that immune effectors can play a significant role in controlling tumor growth under natural conditions or in response to therapeutic manipulation, cancer cells usually evade immune surveillance [3]. Interestingly, innate immunity polypeptides seem to overcome these limitations via a unique mechanism of cancer cell killing that involves membrane lysis [5–7]. These peptides, which are mostly cationic and adopt an amphipathic structure [8–16], were initially discovered due to their role in the clearance of bacteria (for reviews, see [8, 17–19]). They are found in most living species and are released in response to bacterial infection by a different regulatory process [8, 20–22]. Based on their spectrum of activity, these host defense membrane-active peptides can be divided into two major groups. The first group includes peptides that are highly potent against both bacteria and cancer cells but not against normal mammalian cells, e.g. insect cecropins [23, 24] and magainins [25–27] isolated from the skin of frogs and others [7, 28, 29]. The second group includes peptides that are toxic to bacteria and both mammalian cancer and non-cancer cells; some examples include the bee venom melittin [30], tachyplesin II isolated from the horseshoe crab [10, 31], human neutrophil defensins [32, 33], insect defensins [34, 35] and the human LL-37 [36, 37]. Nevertheless, many antimicrobial peptides do not possess antitumor activity [7, 27, 38–40], which raises two major questions: (i) How can different

* Corresponding author.

types of cells have different susceptibilities toward a certain host defense peptide? And (ii) what is the molecular explanation in terms of the peptides' properties that are needed for their cell specificity?

How do cancer and non-cancer cells differ, making the former more susceptible to cytolytic peptides?

Host defense peptides that disrupt target cell membranes (cytoplasmic and/or mitochondrial) as part of the killing effect cause irreversible damage [31, 41–44]. Therefore, studies have focused on identifying the differences between the cellular membranes of the target cells [13–16, 24, 45, 46]. Compared with the highly negatively charged outer surface of bacterial and mitochondrial membranes, the outer membrane of cancer cells contains only a small amount of negatively charged phosphatidylserin (PS) (3–9% of the total membrane phospholipids), being only slightly more negative than that of normal eukaryotic cells [24, 47]. Despite this, some cationic antimicrobial peptides are more toxic to cancer cells than to normal cells [48]. Recent studies have suggested only a partial role for the increased PS found on the outer surface of cancer cells in the selective cytotoxicity of the peptides, compared with a major role of the negative charge in the membrane of bacteria [7, 38]. Note that the membranes of many cancer cells also contain O-glycosylated mucines (high molecular weight glycoproteins consisting of a backbone protein to which oligosaccharides are attached via the hydroxyl groups of serine or threonine [49]). These glycoproteins create an additional negative charge on the cancer cell's surface. Furthermore, we cannot rule out the possibility that the higher negative potential within cancer cells, compared with that of non-cancer cells, also contributes to the selective lytic activity of antimicrobial peptides [27]. Another plausible explanation for the different susceptibilities of normal and cancer cells to cytolytic peptides is based on the relatively higher number of microvilli on tumorigenic cells compared with normal cells [48]. This consequently increases the surface area of the tumorigenic cell membranes and enables binding of a larger amount of the peptides [50].

Different mechanisms for cell killing

Host defense peptides can trigger necrosis via the cell membrane lytic effect

Most membrane-active peptides bind rapidly to the plasma membrane of cancer cells and disrupt it, and as a result the cell dies. The existence of such a membranolytic mechanism was first proved in a study of the antimicrobial peptide magainin and its synthetic analogues, which were active against hematopoietic and solid tumors at concentrations that are relatively nontoxic to well-differentiated normal cells [27]. Importantly, the cytotoxic activity of the analogues against tumor cells was abolished when the electrical gradient across the plasma membrane was eliminated. This demonstrated that the membrane potential is crucial for the disrupting activity of the peptides. Further support for the membranolytic effect comes from the finding that host defense peptides act via a non-receptor-mediated pathway against the target cell membranes; D-amino acid peptide analogues of melittin (a non-cell-selective α-helical lytic peptide), cecropin (a non-hemolytic α-helical peptide active mainly on Gram-positive bacteria), magainin (a non-hemolytic α-helical peptide active on both Gram-positive and Gram-negative bacteria), androctonin (a non-hemolytic β-sheeted peptide containing cysteines) and others displayed activity similar to the all L-amino acid parental peptides [51–55].

Studies of the mode of action of antimicrobial peptides were conducted with de novo-designed membrane-active peptides containing both D- and L-amino acids (termed diastereomers) against several types of cancer cells. The data revealed that the cells died after they were seriously injured, indicating a necrotic pathway [7]. The finding that these peptides depolarized the transmembrane potential of cancer cells at the same rate (within minutes) and concentration at which they showed biological activity suggests the existence of a killing mechanism that indeed involves perturbation of the plasma membrane [38]. Thus these studies support a 'carpet' mechanism for membrane lysis by this group of diastereomeric peptides, which acts similarly to many other native and de novo-designed antimicrobial peptides [11, 15, 55, 56]) (fig. 1). According to the 'carpet' mechanism, the positively charged peptides first associate with the cell membrane through electrostatic interactions and cover it in a carpet-like manner. In the second step, after a threshold concentration has been reached, the peptides insert into the membrane and permeate it. Continuous membrane permeation can lead to micellization. An early step before the collapse of the membrane packing may include the formation of transient holes in the membrane. Such holes are termed torodial pores when the peptide is long enough to span the membrane [57, 58]. Alternatively, lytic peptides can bind via hydrophobic interactions with membranes to form transmembrane channels/pores via the 'barrel-stave' mechanism (fig. 1) [59, 60]. However, in contrast to peptides that act via the carpet mechanism, such peptides have been shown to be non-cell-selective and also lyse normal cells [43, 61, 62].

Host defense peptides can trigger apoptosis via mitochondrial membrane disruption

If internalized inside eukaryotic cells, membrane-active peptides can induce the permeation and swelling of mitochondria, resulting in the release of cytochrome *c,* which

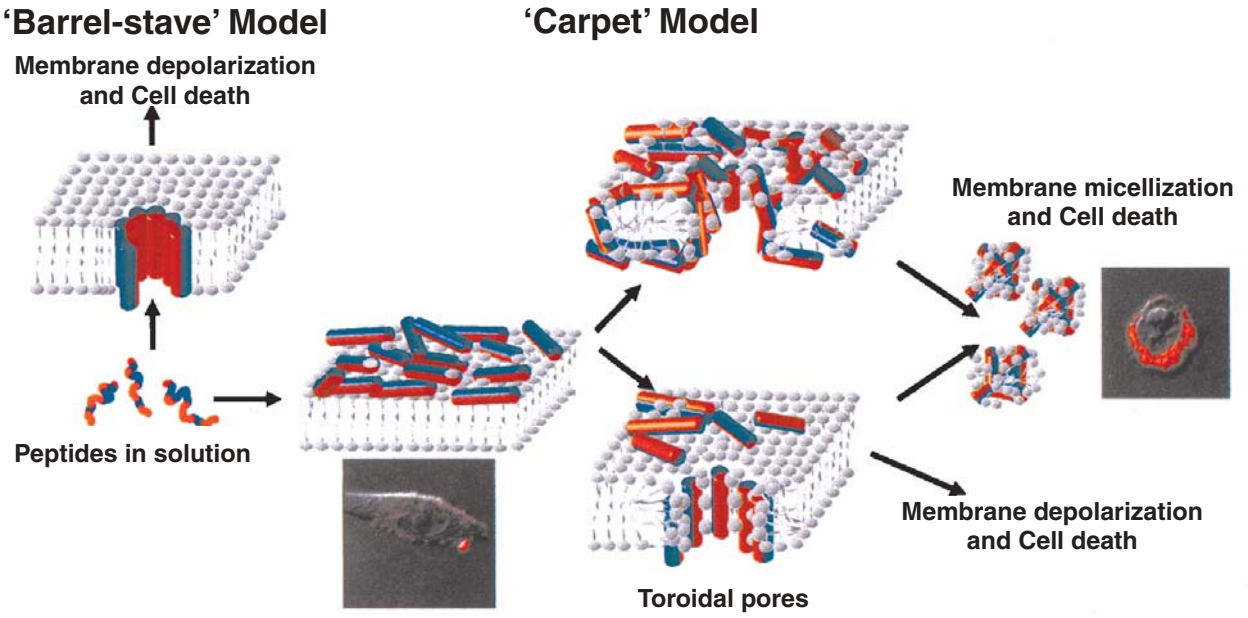

Figure 1. A cartoon illustrating the different mechanisms of membrane lysis by host defense peptides. Peptides that are not cell-selective bind to all types of membranes and form transmembrane pores via the 'barrel-stave' mechanism [59]. This results in membrane depolarization followed by cell death. Peptides that are cancer cell-selective bind in the first step mainly by electrostatic interactions and align parallel to the outer membrane surface (step 1) and cover it in 'carpet-like' manner [55, 86]. After a threshold concentration of peptides has been reached (step 2), the peptides permeate the membrane, which, in most cases, is followed by membrane disintegration and micellization. An intermediate step is the formation of transient pores. These pores were described as 'toroidal' pores for peptides that are long enough to span the membrane [57, 58]. These pores might also lead directly to cell death. The figure also shows B16 melanoma cells treated with a lytic peptide labeled with rhodamine before (picture on the left showing the intact cell) and after (pictures on the right showing the remaining nucleus) a threshold concentration has been reached.

leads to apoptosis [31, 63]. The release of cytochrome *c* from damaged mitochondria induces Apaf-1 oligomerization, caspase 9 activation and subsequently the conversion of pro-caspase 3 to caspase 3, which is responsible for many of the hallmarks of apoptotic symptoms [64]. For example, Ellerby et al. [41] reported on a cationic membrane-active antimicrobial peptide (KLAKLAKKLAKLAK) fused to a CNGRC homing domain that exhibits antitumor activity by targeting mitochondria and triggering apoptosis. In another study, granulysin, a cytolytic molecule released by CTL via granule-mediated exocytosis, was shown to depolarize the mitochondrial membrane potential and to induce tumoral cell death via the mitochondrial pathway of apoptosis [65].

In addition to the mitochondrial pathway, the induction of apoptosis in cancer cells can also be associated with the death receptor pathway. Both pathways were demonstrated in the heptadeca cationic antimicrobial peptide tachyplesin, which is conjugated to RGD, an integrin homing domain [66, 67]. The peptide could interact with the mitochondrial membranes of cancer cells, and the involvement of the mitochondrial pathway was indeed verified [68]. In addition, it was found that members of the death receptor pathway (Fas ligand, FADD and caspase 8) were also up-regulated. Thus, RGD-tachyplesin has more than one effect on cancer cells.

Non-membranolytic modes of action

Direct disruption of mitochondrial or plasma membranes in cancer cells is not the only mechanism by which cytolytic peptides can kill cancer cells. For example, melittin was found to specifically counter-select for mammalian cells in culture that expressed high levels of the *ras* oncogen [69, 70]. Melittin was shown to preferentially hyperactivate phospholipase A2 (PLA2) in *ras* oncogene-transformed cells, resulting in their selective destruction. In another study, melittin and cecropin (either as intact peptides or when expressed in cells using retroviral expression plasmids) were shown to reduce human immunodeficiency virus 1 (HIV-1) replication and gene expression in acutely infected cells at subtoxic concentrations. This was accomplished by decreased levels of Gag antigen and HIV-1 messenger RNAs (mRNAs). Transient transfection assays with HIV long terminal repeat (LTR)-driven reporter gene plasmids indicated that melittin has a direct suppressive effect on the activity of the HIV LTR. It was concluded that these peptides are capable of inhibiting cell-associated production of HIV-1 by suppressing HIV-1 gene expression [71]. Such a transcriptional inhibitory effect is probably due to an indirect mechanism, which is mediated, for example, by the ability of the cytolytic peptide to interfere with signal transduction pathways [72].

Another indirect mechanism was suggested for the antitumor activity of the insect antimicrobial peptide alloferon. The peptide induced immunomodulatory effects that resulted in antitumor resistance in mice. These include stimulating the natural cytotoxicity (NK cells) of human peripheral blood lymphocytes, as well as interferon (IFN) synthesis in both mice and human models [73]. These observations may imply that, in addition to their being active on their own, cytolytic peptides may activate or act synergistically with other host defense components in order to clear tumors.

In vivo studies

How to translate in vitro to in vivo activity?

A major obstacle is to translate the in vitro to in vivo activity. The antitumor potential of magainins observed in the in vitro experiments led to a series of in vivo studies designed to investigate their therapeutic potential [26]. Mice bearing several types of tumors [administered intraperitoneally (i.p.)] were i.p.-injected with magainin 2 and its all-D amino acid analogue MSI-238, both of which were active against P388 leukemia, S180 ascites and a spontaneous ovarian tumor. MSI-238 was more potent, presumably because of its lower susceptibility to enzymatic degradation. However, whereas MSI-238 was ~10-fold more active than magainin in vitro, it was only ~2-fold more active in vivo against murine tumors.

To overcome problems regarding the peptides' proteolysis or toxicity, different strategies were developed [7, 31, 66, 72]. Winder et al. used vector-mediated delivery of genes encoding cell lytic and antimicrobial peptides into tumor cells. Expression constructs carrying cecropin or melittin were introduced into a human bladder carcinoma-derived cell line, and the resulting cell clones were analyzed for tumorigenicity in nude mice. Expression of cecropin resulted in either a complete loss of tumorigenicity in some clones or reduced tumorigenicity in others. Although it is still a challenge to target tumor cell genes encoding antimicrobial peptides, such a strategy represents an important step toward using lytic peptides in cancer therapy.

Another strategy to decrease the toxicity of the peptides is to target them to specific sites by using homing domains. Two examples are discussed: (i) The proapoptotic DP1 peptide, which is composed of a protein transduction domain fused to an antimicrobial peptide $(KLAKLAK)_2$, was able to trigger rapid apoptosis in a variety of cell lines in vitro and in mice xenografts when injected locally. The peptide was active against mitochondrial membranes but was not active on membranes of normal cells. (ii) RGD-tachyplesin inhibited the proliferation of cultured B16 melanoma and TSU prostate cancer cells in vitro, and also inhibited the growth of the same tumors (xenografts) in chicken CAM (topical treatment) and mouse models [subcutaneous (s.c.) injection] [66]. Despite its lytic effect, the peptide did not show toxicity.

The in vivo potency of the peptides is also governed by factors such as serum. The serum components albumin and high-density lipoprotein have only slight inhibitory properties, but the low-density lipoprotein is a potent inhibitor of peptide-mediated cell lysis [74]. For example, the antitumor activity of human defensins is completely abolished by low levels of serum [33]. In order to overcome both serum inactivation and toxicity toward healthy cells, a series of studies were undertaken using de novo-designed antimicrobial peptides composed of both D and L amino acids (termed diastereomers) [38]. One specific diastereomer (15 amino acids long), which showed high selectivity against B16 melanoma and 3LL-D122 lung carcinoma cells in culture, was also active in inhibiting melanoma and lung metastasis in mice [7]. In fact, it decreased the lung metastatic load in C57BL/6 mice by 86% without causing any noticeable weakness or loss of body weight in the animals. A histopathological evaluation revealed that the diastereomer, although given intravenously, did not cause damage to any of the mice's organs. In a recent study, another diastereomeric analogue targeted both androgen-independent (AI) and dependent (AD) human prostate carcinoma (PC) cell lines (CL1, 22RV1 and LNCaP). In addition, a complete growth arrest and a significant lowering of the prostate-specific antigen (PSA) serum levels were observed in prostate tumor xenografts intratumorally treated with the diastereomer [75]. In contrast, the parental all L-amino acid peptide, which was highly active in vitro, could not discriminate between tumor and non-tumor cells and lost its activity in animal models, probably owing to serum inactivation.

Synergism and multi-drug resistance (MDR)

The new and unique mode of action of antimicrobial peptides triggered studies of their synergistic effect with conventional chemotherapeutics. For example, cecropins, which are active against a variety of cancer cells (ovarian carcinoma, breast carcinoma and leukemia cells [24]), could synergize with conventional chemotherapeutics (such as S-fluorouracil, cytarabine and cytarabine) against acute lymphoblastic leukemia cells [76].

The drastic destruction effect of most lytic peptides on the cellular membrane should make it more difficult for cancer cells to develop resistance, compared with conventional chemotherapeutic agents. For example, the high potency of magainin analogues against six small cell lung cancer (SCLC) cell lines was not affected by the *mdr*1 gene. The peptides also showed additive antitumor effects when combined with standard chemotherapeutic agents (cisplatin, etoposide and doxorubicin) [77]. Lincke et al.

[78] also found no differences in the IC_{50} (inhibitory concentration 50%) of the magainin 2 analogue against human BRO melanoma cells transfected with the *mdr*1 gene and the parent BRO cells. Since other cell lines may display a different pattern of drug resistance, it is clear that other mechanisms or cell type-specific factors may modulate resistance.

Concluding remarks

The mechanism by which host defense peptides kill cells is poorly understood in most cases. In particular, the role of the composition of the lipid bilayers of the cells in the biological action of these peptides remains controversial. There are several ways in which membranes may be involved in the action of these peptides. The peptides may form pores in the membrane, allowing leakage of ions and other materials from the cell [79–84]. Alternatively, the peptides may significantly disrupt membranes (cell membranes or mitochondrial membranes) via the 'carpet' mechanism [55, 85].

There is no question that, with increasing resistance against conventional chemotherapy, cationic anticancer peptides, as a novel anticancer agent, have potentially desirable features. In particular, they have a broad spectrum of activity, kill cancer cells rapidly, are unaffected by classical chemotherapy resistance mutations, do not easily select chemotherapy-resistant variants, show synergy with classical chemotherapy, destroy primary tumors, prevent metastases and do not destroy vital organs.

This short manuscript should be regarded as a first attempt to summarize studies on the potential use of host defense peptides as a new family of anticancer drugs with a new mode of action, as well as to correlate between the peptides' mode of action and their target specificity. Although such a correlation is not achieved in all cases, our goal is to inspire investigators to strive to elucidate the parameters that determine peptide activity and specificity toward cancer cells. It is our hope that the rapidly accumulating knowledge in this field will also aid in developing a more rational approach in cancer treatment by cytolytic peptides.

Acknowledgments. The authors are grateful to the Prostate Cancer Foundation (PCF) (former CapCure), the Israel Cancer Research Fund (ICRF) and the Israel Cancer Association (ICA) for generous financial support, and apologize to those whose data have been cited indirectly.

1 Smith L. L., Brow K., Carthew P., Lim C. K., Martin E. A., Styles J. et al. (2000) Chemoprevention of breast cancer by tamoxifen: risks and opportunities. Crit. Rev. Toxicol. **30:** 571–594
2 Garnier-Suillerot, A. Marbeuf-Gueye C., Salerno M., Loetchutinat C., Fokt I., Krawczyk M. et al. (2001) Analysis of drug transport kinetics in multidrug-resistant cells: implications for drug action. Curr. Med. Chem. **8:** 51–64
3 Dunn G. P., Bruce A. T., Ikeda H., Old L. J. and Schreiber R. D. (2002) Cancer immunoediting: from immunosurveillance to tumor escape. Nat. Immunol. **3:** 991–998
4 Mocellin S., Rossi C. R. and Nitti D. (2004) Cancer vaccine development: on the way to break immune tolerance to malignant cells. Exp. Cell Res. **299:** 267–278
5 Shadidi M. and Sioud M. (2003) Selective targeting of cancer cells using synthetic peptides. Drug Resist. Updat. **6:** 363–371
6 Leuschner, C. and Hansel, W. (2004) Membrane disrupting lytic peptides for cancer treatments. Curr. Pharm. Des. **10:** 2299–2310
7 Papo N., Shahar M., Eisenbach L. and Shai Y. (2003) A novel lytic peptide composed of D, L amino acids selectively kills cancer cells in culture and in mice. J. Biol. Chem. **278:** 21018–21023
8 Boman H. G. (1995) Peptide antibiotics and their role in innate immunity. Annu. Rev. Immun. **13:** 61–92
9 Breukink E. and de Kruijff B. (1999) The lantibiotic nisin, a special case or not? Biochim. Biophys. Acta **1462:** 223–234
10 Dimarcq J. L., Bulet P., Hetru C. and Hoffmann J. (1998) Cysteine-rich antimicrobial peptides in invertebrates. Biopolymers **47:** 465–477
11 Shai Y. (1999) Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by alpha-helical antimicrobial and cell non-selective membrane-lytic peptides [Review]. Biochim. Biophys. Acta **1462:** 55–70
12 Tossi A., Sandri L. and Giangaspero A. (2000) Amphipathic, alpha-helical antimicrobial peptides. Biopolymers **55:** 4–30
13 Steiner H., Andreu D. and Merrifield R. B. (1988) Binding and action of cecropin and cecropin analogues: antibacterial peptides from insects. Biochim. Biophys. Acta **939:** 260–266
14 Matsuzaki K., Harada M., Handa T., Funakoshi S., Fujii N., Yajima H. et al. (1989) Magainin 1-induced leakage of entrapped calcein out of negatively-charged lipid vesicles. Biochim. Biophys. Acta **981:** 130–134
15 Pouny Y., Rapaport D., Mor A., Nicolas P. and Shai Y. (1992) Interaction of antimicrobial dermaseptin and its fluorescently labeled analogues with phospholipid membranes. Biochemistry **31:** 12416–12423
16 Gazit E., Lee W. J., Brey P. T. and Shai Y. (1994) Mode of action of the antibacterial cecropin B2: a spectrofluorometric study. Biochemistry **33:** 10681–10692
17 Ganz T. and Lehrer R. I. (1998) Antimicrobial peptides of vertebrates. Curr. Opin. Immunol. **10:** 41–44
18 Simmaco M., Mignogna G. and Barra D. (1998) Antimicrobial peptides from amphibian skin: what do they tell us? Biopolymers **47:** 435–450
19 Zasloff M. (2002) Antimicrobial peptides of multicellular organisms. Nature **415:** 389–395
20 Nicolas P. and Mor A. (1995) Peptides as weapons against microorganisms in the chemical defense system of vertebrates. Annu. Rev. Microbiol. **49:** 277–304
21 Hancock R. E. and Diamond G. (2000) The role of cationic antimicrobial peptides in innate host defences. Trends Microbiol. **8:** 402–410
22 Brotz, H and Sahl H. G. (2000) New insights into the mechanism of action of lantibiotics-diverse biological effects by binding to the same molecular target. J. Antimicrob. Chemother. **46:** 1–6
23 Steiner H., Hultmark D., Engstrom A., Bennich H. and Boman H. G. (1981) Sequence and specificity of two antibacterial proteins involved in insect immunity. Nature **292:** 246–248
24 Chen H. M., Wang W., Smith D. and Chan S. C. (1997) Effects of the anti-bacterial peptide cecropin B and its analogs, cecropins B-1 and B-2, on liposomes, bacteria and cancer cells. Biochim. Biophys. Acta **1336:** 171–179
25 Zasloff M. (1987) Magainins, a class of antimicrobial peptides from *Xenopus* skin: isolation, characterization of two active forms and partial cDNA sequence of a precursor. Proc. Natl. Acad. Sci. USA **84:** 5449–5453

26 Baker M. A., Maloy W. L., Zasloff M. and Jacob L. S. (1993) Anticancer efficacy of Magainin2 and analogue peptides. Cancer Res. **53:** 3052–3057
27 Cruciani R. A., Barker J. L., Zasloff M., Chen H. C. and Colamonici O. (1991) Antibiotic magainins exert cytolytic activity against transformed cell lines through channel formation. Proc. Natl. Acad. Sci. USA **88:** 3792–3796
28 Rozek T., Wegener K. L., Bowie J. H., Olver I. N., Carver J. A., Wallace J. C. et al. (2000) The antibiotic and anticancer active aurein peptides from the Australian Bell Frogs *Litoria aurea* and *Litoria raniformis* the solution structure of aurein 1.2. Eur. J. Biochem. **267:** 5330–5341
29 Papo N., Oren Z., Pag U., Sahl H. G. and Shai Y. (2002) The consequence of sequence alteration of an amphipathic alpha-helical antimicrobial peptide and its diastereomers. J. Biol. Chem. **277:** 33913–33921
30 Habermann E. and Jentsch J. (1967) Hoppe Seyler's Z. Physiol. Chem. **348:** 37–50
31 Mai J. C., Mi Z., Kim S. H., Ng B. and Robbins P. D. (2001) A proapoptotic peptide for the treatment of solid tumors. Cancer Res. **61:** 7709–7712
32 Andreu D. and Rivas L. (1998) Animal antimicrobial peptides: an overview. Biopolymers **47:** 415–433
33 Lichtenstein A., Ganz T., Selsted M. E. and Lehrer R. I. (1986) In vitro tumor cell cytolysis mediated by peptide defensins of human and rabbit granulocytes. Blood **68:** 1407–10.
34 Vizioli J. and Salzet M. (2002) Antimicrobial peptides versus parasitic infections? Trends Parasitol. **18:** 475–476
35 Bulet P., Hetru C., Dimarcq J. L. and Hoffmann D. (1999) Antimicrobial peptides in insects; structure and function. Dev. Comp. Immunol. **23:** 329–344
36 Frohm M., Agerberth B., Ahangari G., Stahle-Backdahl M., Liden S., Wigzell H. et al. (1997) The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. J. Biol. Chem. **272:** 15258–15263
37 Johansson J., Gudmundsson G. H., Rottenberg M. E., Berndt K. D. and Agerberth B. (1998) Conformation-dependent antibacterial activity of the naturally-occuring human peptide LL-37. J. Biol. Chem. **273:** 3718–3724
38 Papo N. and Shai Y. (2003) New lytic peptides based on the D, L amphipathic helix motif preferentially kill tumor cells compared to nornal cells. Biochemistry **42:** 9346–9354
39 Shin S. Y., Kang S. W., Lee D. G., Eom S. H., Song W. K. and Kim J. I. (2000) CRAMP analogues having potent antibiotic activity against bacterial, fungal and tumor cells without hemolytic activity. Biochem. Biophys. Res. Commun. **275:** 904–909
40 Srisailam S., Arunkumar A. I., Wang W., Yu C. and Chen H. M. (2000) Conformational study of a custom antibacterial peptide cecropin B1: implications of the lytic activity. Biochim. Biophys. Acta **1479:** 275–285
41 Ellerby H. M., Arap W., Ellerby L. M., Kain R., Andrusiak R., Rio G. D. et al. (1999) Anti-cancer activity of targeted pro-apoptotic peptides. Nat. Med. **5:** 1032–1038
42 Lehrer R. I. and Ganz T. (1999) Antimicrobial peptides in mammalian and insect host defence. Curr. Opin. Immunol. **11:** 23–27
43 Shai Y. (2002) Mode of action of membrane active antimicrobial peptides. Biopolymers **66:** 236–248
44 Hoffmann J. A., Kafatos F. C., Janeway C. A. and Ezekowitz R. A. (1999) Phylogenetic perspectives in innate immunity. Science **284:** 1313–1318
45 Hendrich A. B. and Michalak K. (2003) Lipids as a target for drugs modulating multidrug resistance of cancer cells. Curr. Drug Targets **4:** 23–30
46 Papo N. and Shai Y. (2003) Can we predict biological activity of antimicrobial peptides from their interactions with model phospholipid membranes? Peptides **24:** 1693–1703
47 Verkleij A. J., Zwaal R. F., Roelofsen B., Comfurius P., Kastelijn D. and Deenen L. V. (1973) The asymmetric distribution of phospholipids in the human red cell membrane. A combined study using phospholipases and freeze-etch electron microscopy. Biochim. Biophys. Acta **323:** 178–193
48 Zwaal R. F. and Schroit A. J. (1997) Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. Blood **89:** 1121–1132
49 Cappelli G., Paladini S. and D'Agata A. (1999) Tumor markers in the diagnosis of pancreatic cancer. Tumori **85:** S19–21.
50 Chan S. C., Hui L. and Chen H. M. (1998) Enhancement of the cytolytic effect of anti-bacterial cecropin by the microvilli of cancer cells. Anticancer Res. **18:** 4467–4474
51 Wade D., Boman A., Wahlin B., Drain C. M., Andreu D., Boman H. G. et al. (1990) All-D amino acid-containing channel-forming antibiotic peptides. Proc. Natl. Acad. Sci. USA **87:** 4761–4765
52 Bessalle R., Kapitkovsky A., Gorea A., Shalit I. and Fridkin M. (1990) All-D-magainin: chirality, antimicrobial activity and proteolytic resistance. FEBS Lett. **274:** 151–155
53 Merrifield R. B., Juvvadi P., Andreu D., Ubach J., Boman A. and Boman H. G. (1995) Retro and retroenantio analogs of cecropin-melittin hybrids. Proc. Natl. Acad. Sci. USA **92:** 3449–3453
54 Hetru C., Letellier L., Oren Z., Hoffmann J. A. and Shai Y. (2000) Androctonin, a hydrophilic disulphide-bridged non-haemolytic anti-microbial peptide: a plausible mode of action. Biochem. J. **345:** 653–664
55 Oren Z. and Shai Y. (1998) Mode of action of linear amphipathic alpha-helical antimicrobial peptides. Biopolymers **47:** 451–463
56 Papo N. and Shai Y. (2003) Exploring peptide membrane interaction using surface plasmon resonance: differentiation between pore formation versus membrane disruption by lytic peptides. Biochemistry **42:** 458–466
57 Matsuzaki K., Murase O. and Miyajima K. (1995) Kinetics of pore formation by an antimicrobial peptide, magainin 2, in phospholipid bilayers. Biochemistry **34:** 12553–12559
58 Ludtke S. J., He K., Heller W. T., Harroun T. A., Yang L. and Huang, H. W. (1996) Membrane pores induced by magainin. Biochemistry **35:** 13723–13728
59 Ehrenstein G. and Lecar H. (1977) Electrically gated ionic channels in lipid bilayers. Q. Rev. Biophys. **10:** 1–34
60 Vogel H., Jahnig F., Hoffmann V. and Stumpel J. (1983) The orientation of melittin in lipid membranes. A polarized infrared spectroscopy study. Biochim. Biophys. Acta **733:** 201–209
61 Shai Y., Bach D. and Yanovsky A. (1990) Channel formation properties of synthetic pardaxin and analogues. J. Biol. Chem. **265:** 20202–20209
62 Shai Y. and Oren Z. (1996) Diastereoisomers of cytolysins, a novel class of potent antibacterial peptides. J. Biol. Chem. **271:** 7305–7308
63 Li H., Kolluri S. K., Gu J., Dawson M. I., Cao X., Hobbs P. D. et al. (2000) Cytochrome c release and apoptosis induced by mitochondrial targeting of nuclear orphan receptor TR3. Science **289:** 1159–1164
64 Cory S. and Adams J. M. (1998) Matters of life and death: programmed cell death at Cold Spring Harbor. Biochim. Biophys. Acta **1377:** R25–44
65 Pardo J., Perez-Galan P., Gamen S., Marzo I., Monleon I., Kaspar A. A. et al. (2001) A role of the mitochondrial apoptosis-inducing factor in granulysin-induced apoptosis. J. Immunol. **167:** 1222–1229
66 Chen, Y. Xu X., Hong S., Chen J., Liub N., Underhill C. B. et al. (2001) RGD-tachyplesin inhibits tumor growth. Cancer Res. **61:** 2434–2438
67 Park N. G., Lee S., Oishi O., Aoyagi H., Iwanaga S., Yamashita S. et al. (1992) Conformation of tachyplesin I from *Tachypleus tridentatus* when interacting with lipid matrices. Biochemistry **31:** 12241–12247

68 Kaufmann S. H. and Earnshaw W. C. (2000) Induction of apoptosis by cancer chemotherapy. Exp. Cell Res. **256:** 42–49
69 Rasmussen H. R. J. E. (1990), vol. 31, Horecker B. L., Stadtman E. R., Chock P. B. and Levitski A., eds, pp. 1–98, Academic Press, San Diego
70 Sharma S. V. (1992) Melittin resistance: a counterselection for ras transformation. Oncogene **7:** 193–201
71 Wachinger M., Kleinschmidt A., Winder D., von Pechmann N., Ludvigsen A., Neumann M. et al. (1998) Antimicrobial peptides melittin and cecropin inhibit replication of human immunodeficiency virus 1 by suppressing viral gene expression. J. Gen. Virol. **79:** 731–740
72 Winder D., Gunzburg W. H., Erfle V. and Salmons B. (1998) Expression of antimicrobial peptides has an antitumour effect in human cells. Biochem. Biophys. Res. Commun. **242:** 608–612
73 Chernysh S., Kim S. I., Bekker G., Pleskach V. A., Filatova N. A., Anikin, V. B. et al. (2002) Antiviral and antitumor peptides from insects. Proc. Natl. Acad. Sci. USA **99:** 12628–12632
74 Peck M. K., Darveau R. P. and Fell H. P. (1993) Identification of serum components that inhibit the tumoricidal activity of amphiphilic alpha helical peptides. Cancer Chemother. Pharmacol. **32:** 109–115
75 Papo N., Braunstein A., Eshhar Z. and Shai Y. (2004) Suppression of human prostate tumor growth in mice by a cytolytic D-, L-amino acid peptide: membrane lysis, increased necrosis and inhibition of prostate-specific antigen secretion. Cancer Res. **64:** 5779–5786
76 Hui L., Leung K. and Chen H. M. (2002) The combined effects of antibacterial peptide cecropin A and anti-cancer agents on leukemia cells. Anticancer Res. **22:** 2811–2816
77 Ohsaki Y., Gazdar A. F., Chen H. C. and Johnson B. E. (1992) Antitumor activity of magainin analogues against human lung cancer cell lines. Cancer Res. **52:** 3534–3538
78 Lincke C. R., van der Bliek A. M., Schuurhuis G. J., van der Velde-Koerts T., Smit J. J. and Borst P. (1990) Multidrug resistance phenotype of human BRO melanoma cells transfected with a wild–type human mdr1 complementary DNA. Cancer Res. **50:** 1779–1785
79 Vogel H. and Jahnig F. (1986) The structure of melittin in membranes. Biophys. J. **50:** 573–582
80 Beschiaschvili G. and Seelig J. (1990) Melittin binding to mixed phosphatidylglycerol/phosphatidylcholine membranes. Biochemistry **29:** 52–58
81 Schwarz G., Gerke H., Rizzo V. and Stankowski S. (1987) Incorporation kinetics in a membrane, studied with the pore-forming peptide alamethicin. Biophys. J. **52:** 685–692
82 Pawlak M., Kuhn A. and Vogel H. (1994) Pf3 coat protein forms voltage-gated ion channels in planar lipid bilayers. Biochemistry **33:** 283–290
83 Marsh D. (1996) Peptide models for membrane channels. Biochem. J. **315:** 345–361
84 Matsuzaki K., Murase O., Fujii N. and Miyajima K. (1995) Translocation of a channel-forming antimicrobial peptide, magainin 2, across lipid bilayers by forming a pore. Biochemistry **34:** 6521–6526
85 Ladokhin A. S. and White S. H. (2001) 'Detergent-like' permeabilization of anionic lipid vesicles by melittin. Biochim. Biophys. Acta **1:** 253–260
86 Pouny Y. and Shai Y. (1992) Interaction of D-amino acid incorporated analogues of pardaxin with membranes. Biochemistry **31:** 9482–9490