Chapter 4
Anticancer Peptides: Prospective Innovation in Cancer Therapy

Diana Gaspar and Miguel A.R.B. Castanho

Abstract Current cancer treatments require improvements in selectivity and efficacy. Surgery, radiation, and chemotherapy approaches result in patient's suffering over time due to the development of severe side-effects that simultaneously condition adherence to therapy. Biologically active peptides, in particular antimicrobial peptides (AMPs), are versatile molecules in terms of biological activities. The cytotoxic activities of several AMPs turn this group of molecules into an amazing pool of new templates for anticancer drug development. However, several unmet challenges limit application of peptides in cancer therapy. The mechanism(s) of action of the peptides need better description and understanding, and innovative targets have to be discovered and explored, facilitating drug design and development. In this chapter, we explore the natural occurring AMPs as potential new anticancer peptides (ACPs) for cancer prevention and treatment. Their modes of action, selectivity to tumor compared to normal cells, preferential targets, and applications, but also their weaknesses, are described and discussed.

4.1 Introduction

Even though sharing similar characteristics such as replicative immortality, ability to evade immunosurveillance, and ability to invade surrounding and distant tissues and organs (Wu et al. 2014), tumor cells are still a challenging target in oncology. The development of resistance mechanisms and specific contributions of each tumor microenvironment (TME) contribute for the many difficulties in selectively targeting diseased rather than normal cells. These limitations in oncology treatment

D. Gaspar (✉) · M.A.R.B. Castanho
Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa,
Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal
e-mail: dianagaspar@medicina.ulisboa.pt

M.A.R.B. Castanho
e-mail: macastanho@medicina.ulisboa.pt

are the main reason why cancer diseases remain a leading cause of death worldwide.

The term cancer refers to a group of diseases characterized by an uncontrolled growth and by the spread of abnormal cells (Sah et al. 2015). The carcinogenesis process, triggered either by external factors such as radiation or chemicals, but also by internal factors such as mutations and hormones (Tanaka 1997), encompasses many changes on the cells' biochemistry (Tanaka 2009). Advances in oncotherapy need to address the particular biochemical signatures of each tumor. Identifying these signatures and exploiting their vulnerabilities will lead to the development of selective anticancer drugs than can prolong patients' lifetime and delay or prevent tumor metastases.

Conventional therapies including chemotherapy fail in selecting effectively which cells are to be targeted. While delivering a cytotoxic compound to the tumor, either a DNA-alkylating agent or a hormone agonist/antagonist, several constraints determine the fate of both malignant and normal cells. These include the effective localization of the chemotherapeutic drug, but also drug's biodistribution and selectivity determinants (Chen et al. 2014). These therapeutic options have been successful in converting some fatal cancers into chronic diseases that allow patients to survive for many years. However, the secondary effects that eventually arise in this process result in patients' suffering and slow clinical status deterioration with stages of myelosuppression, thrombocytopenia, mucositis, and alopecia (Riedl et al. 2011) before culminating in death.

In this scenario, peptide-based drugs raise renewed hope (Wu et al. 2014). The development of peptide sequences designed to interact with specific molecular markers, receptors or other tumor cell components, has been of value for application in cancer diagnosis, prognosis, and treatment. In this chapter we will review the use of peptides on cancer treatment, with focuses in their natural sources and specificity of their mechanism(s) of action.

4.2 Peptide-Based Strategies for Cancer Treatment—Anticancer Peptides

Peptide-based therapies have many benefits for cancer chemotherapy or supportive care, such as low cytotoxicity, strong specificity, tumor-penetrating ability, small size, and ease of modification (Barras and Widmann 2011; Wu et al. 2014). In fact, peptides have small to intermediate sizes, up to just a few hundreds of amino acids residues, amenable pharmacokinetic profiles, high uptake into tissues, and rapid clearance from blood (Wu et al. 2014). Thus, peptides recognizing and binding to specific membrane proteins or receptors on tumor cells' membranes are potential alternative drugs to overcome the limitations of low tissue penetration and low cellular uptake when using monoclonal antibodies (mAbs), for instance (Wu et al. 2014). Furthermore, peptide's production is of lower complexity when compared to

other protein-based therapies and thus, cost-effective (Fosgerau and Hoffmann 2015).

Antimicrobial peptides (AMPs) are a class of natural occurring peptides with several important targets and activities, from antimicrobial, antiviral, and antifungal (Reddy et al. 2004; Torcato et al. 2013a, b; Mello et al. 2011) to the modulation of the immune response (Silva et al. 2012). As part of immune defense (Iwasaki et al. 2009), AMPs are found in eukaryotic organisms of many different species (Reddy et al. 2004) and their rapid and non-specific interactions with the membrane lipids of the microbial targets results in the pathogen death with very low chance of resistance development (Arouri et al. 2009; Fernebro 2011). This interaction is enhanced by the high proportion of cationic and hydrophobic amino acid residues present in the structure of the peptides (Seo et al. 2012). AMPs are electrostatically attracted to the anionic membrane of the microbe and subsequently insert and disrupt the lipid structures, leading to its permeation (Huang et al. 2014). The changes that the cell machinery should endure for producing a resistant biological membrane, capable of neutralizing the action of AMPs is significant biological effort that has been rarely met until today (Chen et al. 2014).

In addition to their antimicrobial properties, some natural and synthetic AMPs also have antitumor activities with varying degrees of selectivity towards cancer cells (Hoskin and Ramamoorthy 2008). In fact, some of these newly found anti-cancer peptides (ACPs) have been successful in decreasing the burden of tumors in many animal models (Bhutia and Maiti 2008; Papo and Shai 2005).

The use of ACPs in oncology has been researched either to treat the tumor directly or to prevent formation of metastases; in this way, they are potential alternatives or adjuvant to the current therapies. Peptides can be used as drugs, hormones, or immunization agents (vaccines) (Sah et al. 2015). The biological effects include inhibition of tumor vasculature growth (angiogenesis), alterations in protein–protein interaction, changes in gene expression, and apoptosis, among others (Rosca et al. 2011; Walensky et al. 2004; Zheng et al. 2011).

The main weaknesses of ACPs are poor stability with susceptibility to proteolytic degradation and insufficient membrane permeability (Craik et al. 2013). There are strategies to overcome these limitations and their consequences (Wu et al. 2014), including amino acid substitution (Kohno et al. 2011), fusion of peptides (Yang et al. 2008), and peptide conjugation with chemotherapeutic drugs (Zhao et al. 2012).

4.3 Mechanisms of Action, Cellular Targets and Selectivity of Anticancer Peptides

There is intensive debate on ACPs' modes of action. Reviews available in the literature provide detailed description of the many different mechanisms underlying cancer cell toxicity (Gaspar et al. 2013; Harris et al. 2013; Hoskin and

Ramamoorthy 2008; Papo and Shai 2005; Mulder et al. 2013). Studies on structure–activity relationship have shown that some ACPs share with AMPs the ability to disrupt cell membranes, causing poration or micellization, and additionally inducing necrosis and/or apoptosis (Bhutia and Maiti 2008; Papo and Shai 2005). Additionally, numerous studies suggest that AMPs and ACPs share similar mechanisms of membrane interaction (Al-Benna et al. 2011; Harris et al. 2013; Riedl et al. 2011). This assumption is supported by the structural requirements that attract AMPs and ACPs to their respective microbial and human cell targets. Other membranolytic effects include mitochondrial swelling with cytochrome c release (Mai et al. 2001). However, non-membranolytic mechanisms are expected to be found for other ACPs (Harris et al. 2013; Sharma 1992) and it is frequent to discover that one ACP can have more than one cellular target and thus follow more than one mode of action. The modes of action not involving direct targeting of the cell membrane, such as interference with nucleic acid synthesis, hormonal receptors, or angiogenesis, have been hypothesized to be part of mediated immunity (Gaspar et al. 2013; Kuriyama et al. 2013).

Short linear ACPs fold into amphipathic conformations upon membrane interaction (Chen et al. 2014; Schweizer 2009), depending on hydrophobicity, amphipathicity, net charge, secondary structure, and oligomerization at the membrane level (Harris et al. 2013; Hoskin and Ramamoorthy 2008). Uncovering the details of the molecular mechanisms underlying each ACP mode of action is a technically challenging but rewarding task because the information gathered from these studies can be successfully applied in the development of innovative approaches in cancer treatment (Medina and Schneider 2015). There are ACPs with high specificity and selectivity for their targets. These include matrix metalloproteinases (MMP) such as MMP-2 and MMP-9 (Koivunen et al. 1999), the c-Src signaling pathway involved in tumor angiogenesis (Yi et al. 2009), cyclooxygenase-2 (Vesely et al. 2006), the heat shock protein 90 (Hsp90) and S100P, a marker for differentiating tumor and normal cells (Sah et al. 2015).

The details of the mechanisms of membrane-targeting ACPs are also not fully elucidated. The cellular membrane in tumors is biochemically modified when compared to normal cells (Huang et al. 2014; Schweizer 2009) because cancer cells have an higher content of anionic lipids in the outer surface of cytoplasmic membrane due to the increased fraction of negatively-charged phospholipids such as phosphatidylserine (PS) (Hoskin and Ramamoorthy 2008; Riedl et al. 2011). The loss of membrane asymmetry in the lipid distribution between the inner and outer leaflet of the plasma membrane during cell transformation into a malignant phenotype appears to be the cause for the exposure of PS on the surface of cells, which contributes to the selectivity of ACPs for solid and non-solid tumors (Gaspar et al. 2013). Other anionic components are also present on cancer cells' membrane such as O-glycosylated mucins, heparin sulfate and sialylated gangliosides (Gaspar et al. 2013). Cholesterol content on tumor cells' membrane also modulates cellular fluidity and condition ACPs activity (Schweizer 2009). The higher transmembrane potential and the higher surface area of tumor cells, which promotes contact with an increased number or peptide molecules, further contribute to the preferred action of

ACPs on tumor cells (Chan et al. 1998; Chaudhary and Munshi 1995; Huang et al. 2014). Peptides such as MPI-1 from the venom wasp *Polybia paulista* (Wang et al. 2009a), NK-2 derived from the protein NK-lysin found in porcines' NK- an T-cells (Schroder-Borm et al. 2005) and the synthetic peptide SVS-1 (Gaspar et al. 2012; Sinthuvanich et al. 2012) are a few examples of natural and synthetic peptides that base their preference for solid and hematological tumor cells based on the membrane surface net charge. However, detailed studies using biophysical and imaging techniques have shown that even though net charge has an important role in determining membrane interaction, AMPs and ACPs tend to behave differently in lipid environment (Freire et al. 2015; Gaspar et al. 2012, 2015). SVS-1 and HNP-1 ACPs are examples of this difference (Fig. 4.1). SVS-1 was designed to adopt a β-sheet structure after contact with the negatively-charged cancer cell membrane and is preferentially cytotoxic against lung, epidermal, and breast carcinomas when compared to HUVEC and red blood cells (Gaspar et al. 2012; Sinthuvanich et al. 2012). The mode of action described for SVS-1 is a lytic mechanism involving cell-surface induced folding into a β-hairpin structure capable of forming pores in the cell membrane (Sinthuvanich et al. 2012) (Fig. 4.1). The trigger for the peptide
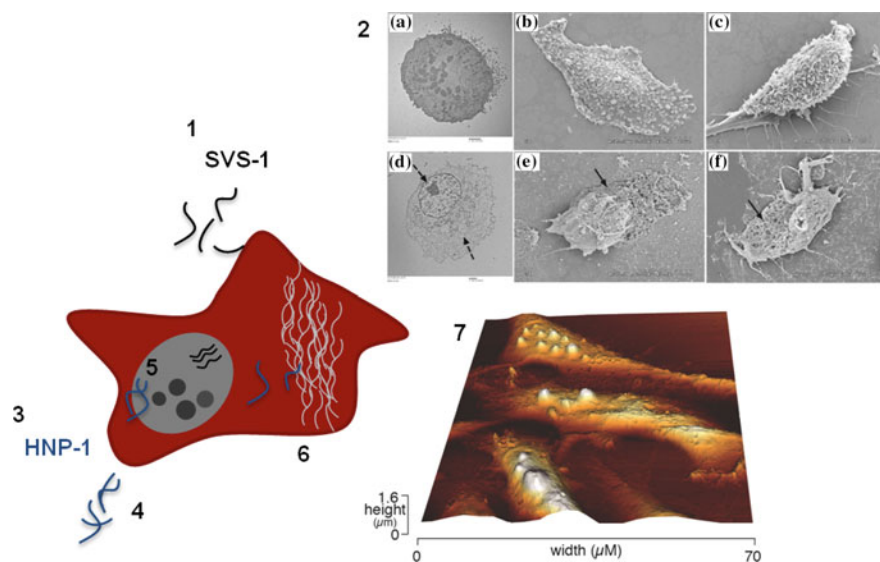
Fig. 4.1 Cell death induced by *SVS-1* and *HNP-1* peptides. *SVS-1* engages electrostatically the cancer cell membrane (*1*) and folds into a β-hairpin structure capable of forming pores (*solid arrow*) with leakage of the cellular contents (*dashed arrow*) as shown by TEM (*2a, 2d*) and SEM (*2c, 2e, 2b, 2f*) images. Scale bar: 10 μm for SEM, 2 μm for TEM. *HNP-1* (*3*) interacts with the negatively-charged cell membrane of the tumor cell (*4*), translocates into the cell-inducing DNA fragmentation (*6*) and fragilizing the cell's cytoskeleton structure (*6*) resulting in the collapse of the cell as shown in the 3D projection of the AFM height image (*7*). For both SVS-1 and HNP-1, cell death precedes membrane neutralization, in contrast to typical AMP action (Alves et al. 2010, Torcato et al. 2013b). Adapted from references Sinthuvanich et al. (2012) and Gaspar et al. (2015)

folding is the membrane net negative charge but full membrane neutralization is not mandatory for cell death (Gaspar et al. 2012). It was recently found that SVS-1 is able to translocate across the cell membrane into the cytoplasm and into the nucleus when present in concentrations below the minimal inhibitory concentration (MIC_{50}) necessary for lytic action (Medina and Schneider 2015). The combination of SVS-1 with paclitaxel improves SVS-1 aqueous solubility and the peptide is capable of delivering and releasing paclitaxel into cancer cells and tumors *in vivo* without any adjuvant (Medina and Schneider 2015).

On the contrary, studies with AMPs such as BP100 show a neutralization of the bacterial membrane that can be correlated with the minimal inhibitory concentration (MIC) values found to inhibit the growth of *Escherichia coli* bacteria (Alves et al. 2010). Therefore, one should be cautious when translating conclusions on AMPs structure-activity studies to ACPs because not all AMPs behave as ACPs.

4.4 Targeting Cancer Cells Using Natural Peptides

More than 7000 natural peptides have been identified until today (Fosgerau and Hoffmann 2015) and AMPs can be found virtually in all living organisms, from plants and insects to animals (Salas et al. 2015). Table 4.1 lists selected examples of these AMPs with anticancer activity.

Natural products derived from plants have contributed greatly to chemotherapy development. Examples of this are the drugs paclitaxel, vincristine, and vinblastine (Wu et al. 2014). Plants are also great producers of small cysteine-rich AMPs and some of them present cytotoxic activities. Cytotoxic classes are mainly represented by thionins, defensins and cyclotides (Guzman-Rodriguez et al. 2015).

Table 4.1 Selected naturally occurring antimicrobial peptides (AMPs) with anticancer activity

Peptide	Source	Activity	Reference
Pyrularia	Plant	Changes in Ca^{2+} influx	Evans et al. (1989)
NaD1	Plant	Binding to plasma membrane PIP2	Poon et al. (2014)
RA-V	Plant	Mitochondria-mediated apoptosis with PDK1-AKT blocking; Inhibition of cell adhesion and migration through regulation of adhesion molecules, receptors and MMPs expression	Fang et al. (2013); Leung et al. (2015)
Lunasin	Plant	HAT inhibition and cell cycle progression repression	Galvez et al. (2001); Hernandez-Ledesma et al. (2009)
Gomesin	Insect	Ca^{2+} accumulation, loss of mitochondrial potential, pore formation	Rodrigues et al. (2008); Paredes-Gamero et al. (20120
Mastoparan	Insect	Oxidative stress, mitochondrial depolarization and apoptosis	de Azevedo (2015)
HNP-1	Human	DNA breakdown and cell collapse	Gaspar et al. (2015)

Thionins are small cysteine-rich peptides with diversified activities, in addition to being antimicrobial. They help seed maturation and germination and have roles in signal transduction (Stec 2006), and some of them, such as pyrularia, Thi2.1, and β-purothionin have cytotoxic activity against cervical, lung, and breast cancers (Evans et al. 1989; Hughes et al. 2000; Loeza-Angeles et al. 2008). In some cases, anticancer effects are based on changes on Ca^{2+} influx that depolarize the cellular membrane (Evans et al. 1989), but for others remain unknown (Loeza-Angeles et al. 2008).

Plant defensins represent a diversified group in terms of their amino acid sequence but some of the amino acid positions are highly conserved (Guzman-Rodriguez et al. 2015). They present powerful antifungal activity (Mello et al. 2011) and their mode of action is related to membrane destabilization or insertion followed by pore formation and leakage of essential biomolecules (Lacerda et al. 2014). Sesquin was the first plant defensin known to be active against breast cancer and leukemia cells (Wong and Ng 2005b). Other plant defensins, such as lunatusin (Wong and Ng 2005a) and phaseococcin (Ngai and Ng 2005) are also active on breast cancer and leukemia; however, their mode of action and selectivity are still poorly described. Several reports on plant defensins show that this group of peptides might have alternative targets to conventional drugs. This is the case of NaD1 that can act by direct binding to the plasma membrane phospholipid phosphatidylinositol 4,5-biphosphate (PIP2) (Poon et al. 2014).

Cyclotides is another group of Cys-rich peptides derived from plants with cytotoxic activity. These macrocyclic peptides have around 30 amino acid residues in their sequence and a wide range of biological activities (Craik 2012). Their tight cyclic structure is of particular relevance because it confers chemical and biological stability, conferring high pharmaceutical value to the peptides (Guzman-Rodriguez et al. 2015). They are characterized by a cystine knot with an embedded ring formed by two disulfide bonds and connecting backbone segments threaded by one more disulfide bond (Guzman-Rodriguez et al. 2015). Expressed in large quantities by plants of Rubiaceae and Violaceae families, cyclotides are described mainly as host protectors (Craik 2012). However, their activities go much further than biocidal protection and include anti-HIV and anticancer effects (Craik 2012). The mechanism of action described for cyclotides is also very interesting from a therapeutical point of view. For kalatas B1–B9 peptides, the presence of phosphatidylethanolamine (PE) headgroups on the cellular membrane favors peptide binding (Henriques et al. 2012), which is advantageous in the drug design process for increasing peptide's selectivity towards specific cancer cells that express higher contents of this PE phospholipid, for instance. Other described cyclotides include cycloviolacin O2 and Viba 15 and 17 with activities against lymphoma, melanoma, and also cervical and gastric cancers (He et al. 2011; Svangard et al. 2007).

More recently, the cyclopeptide deoxybouvardin, RA-V, derived from *Rubia yunnanensis* has been characterized with antitumor and anti-angiogenesis activity (Fang et al. 2013; Leung et al. 2015). This peptide shows anticancer activity against human and murine breast cancer cells through mitochondria-mediated apoptosis by blocking PDK1 and AKT interaction and consequently apoptosis resistance

(Fang et al. 2013). RA-V peptide is also capable of inhibiting breast cancer cell adhesion and migration through the interference on cofilin signaling and chemokine receptors. This peptide reduces the expression of several adhesion molecules and MMPs (Leung et al. 2015).

Lunasin is another example of natural ACP isolated from plants (Hernandez-Ledesma et al. 2009). This 43-amino acid peptide is found in soy, wheat, barley, and other seeds (Hernandez-Ledesma et al. 2009) and is a chemo-preventive agent against oncogenes and chemical carcinogens (Ortiz-Martinez et al. 2014). With an adequate bioavailability following oral administration, lunasin was shown to prevent skin cancer in a mouse model induced by chemical carcinogens (Galvez et al. 2001; Hsieh et al. 2004). An epigenetic mechanism of action proposed for this peptide is the selective killing of newly transformed cancer cells by acting as a histone acetyltransferase (HAT) inhibitor and repressing cell cycle progression (Hernandez-Ledesma et al. 2009).

Insects are also a good natural source of AMPs with anticancer activity. Gomesin is a β-hairpin peptide isolated from the hemolymph of *Acanthoscurria gomesiana*, a Brazilian spider (Rodrigues et al. 2008). This peptide has the ability to form pores and is active as a topical agent against melanoma, breast, and colon carcinomas neuroblastomas and pheochromocytomas (Rodrigues et al. 2008). Gomesin induces membrane permeabilization through a Ca^{2+} dependent pathway which involves particular intracellular events: perturbation of the endoplasmic reticulum, accumulation of Ca^{2+} in organelles, of mitochondrial potential and oxidative stress (Paredes-Gamero et al. 2012). Mastoparan is a 14-amino acid α-helical cell penetrating peptide from the venom of *Vespula lewisii* wasp that has nocive effects on cell membranes (Saar et al. 2005). Mastoparan shows antitumor activity against human erythroleukemia cells and melanoma (Yamada et al. 2005). In the latter, tumor cell death occurs through an induce of programmed cell death by oxidative stress, which causes mitochondrial depolarization (de Azevedo et al. 2015). Apoptosis induced by mastoparan stems from the activation of caspases -9, -12, and -3, cleavage of PARP, up-regulation of pro-apoptotic proteins Bax and Bim and down-regulation of the anti-apoptotic Bcl-XL proteins (de Azevedo et al. 2015).

In animals, AMPs with anticancer activity can be found in the immune, digestive, and central nervous systems (CNS) and also in the heart, bones, muscle, and skin (Wu et al. 2014). Many AMPs from the animal kingdom have been extensively studied, such as LfcinB. This 25-amino acid residues peptide is isolated from cows' milk and causes cell death through at least two mechanisms. LfcinB is active against leukemia cells and diverse solid tumors (Mader et al. 2005) and is capable of binding to glycosaminoglycans (GAGs) present on the membrane surface (Jenssen et al. 2004), inducing apoptosis by mitochondrial pathway, and also lysis of the cellular membrane (Eliassen et al. 2006; Furlong et al. 2008).

One of the most studied groups of peptides derived from humans is the defensins group. These are disulfide-rich peptides, similar to defensin plants, and comprise 29-35 amino acids with three disulfide bonds (Conibear and Craik 2014). Defensins are organized in three classes, α-, β-, and Ѳ-defensins (Conibear and Craik 2014).

The class of α-defensins includes the human neutrophil peptides 1–4 and the human defensins HD5 and HD6, produced in the Paneth cells of the intestine (Ouellette and Bevins 2001). The human neutrophil peptides, HNPs, possess antitumoral effects trough diversified mechanisms (Wang et al. 2009b). HNP-1 to 3 have been appointed has potential tumor biomarkers (Albrethsen et al. 2005, 2006; Droin et al. 2009). The HNP-1 has been intensively studied for anticancer properties. Produced and stored in the azurophilic granules of human neutrophils, this peptide is released when an inactivation of bacteria and yeast is necessary (Ganz and Lehrer 1998). However, many studies report the importance of this AMP in oncology. The expression of HNP-1 in models of tumors such as breast and colon stimulates an immune response from the host against the tumor (Wang et al. 2009c). In addition, it has been found up-regulated in cancers such as colorectal (Mohri et al. 2009) and other tumors (Albrethsen et al. 2006; Holterman et al. 2006) and to be linked to tumor necrosis when expressed intratumorally (Bateman et al. 1992; Muller et al. 2002). HNP-1 mode of action is believed to involve damage to the cell membrane but also the induction of DNA strand break (Gera and Lichtenstein 1991). A recent study revealed that HNP-1 attacks solid tumors, human prostate cancer in this particular case, after translocating into the cell, following DNA and cytoskeleton damage and final cell collapse (Gaspar et al. 2015) (Fig. 4.1). Cell death occurs without full neutralization of the cancer cell membrane and although HNP-1 interacts with prostate and leukemia cells, differences on the membrane composition of each tumor cells dictate the peptide's preference (Gaspar et al. 2015).

4.5 Final Remarks

Today, hundreds of novel peptide sequences are part of the clinical and preclinical testing of pharmaceutical companies. As peptide-based therapies are on the spotlight, a great number of studies report on the role of AMPs on cancer treatment. With a very particular mode of action involving non-specific interactions, peptides can be expected to meet the selectivity, efficacy, and safety requisites for successful drugs with diminutive resistance barriers. However, drug development optimization is still needed, which requires that the mechanism(s) of action and molecular and cellular targets, as well as potential off-target effects, are researched and described with molecular level detail and correlated to human physiology. The future of peptide applications in oncology and oncotherapeutics depends on how successful basic research will be in this endeavor.

References

Al-Benna S, Shai Y, Jacobsen F, Steinstraesser L (2011) Oncolytic activities of host defense peptides. Int J Mol Sci 12(11):8027–8051. doi:10.3390/ijms12118027

Albrethsen J, Bogebo R, Gammeltoft S, Olsen J, Winther B, Raskov H (2005) Upregulated expression of human neutrophil peptides 1, 2 and 3 (HNP 1-3) in colon cancer serum and tumours: a biomarker study. BMC Cancer 5:8. doi:10.1186/1471-2407-5-8

Albrethsen J, Moller CH, Olsen J, Raskov H, Gammeltoft S (2006) Human neutrophil peptides 1, 2 and 3 are biochemical markers for metastatic colorectal cancer. Eur J Cancer 42(17):3057–3064. doi:10.1016/j.ejca.2006.05.039

Alves CS, Melo MN, Franquelim HG, Ferre R, Planas M, Feliu L, Bardaji E, Kowalczyk W, Andreu D, Santos NC, Fernandes MX, Castanho MARB (2010) Escherichia coli cell surface perturbation and disruption induced by antimicrobial peptides BP100 and pepR. J Biol Chem 285(36):27536–27544. doi:10.1074/jbc.M110.130955

Arouri A, Dathe M, Blume A (2009) Peptide induced demixing in PG/PE lipid mixtures: A mechanism for the specificity of antimicrobial peptides towards bacterial membranes? Biochimica Et Biophysica Acta-Biomembranes 1788(3):650–659. doi:10.1016/j.bbamem.2008.11.022

Barras D, Widmann C (2011) Promises of apoptosis-inducing peptides in cancer therapeutics. Curr Pharm Biotechnol 12(8):1153–1165

Bateman A, Singh A, Jothy S, Fraser R, Esch F, Solomon S (1992) The levels and biologic action of the human neutrophil granule peptide Hp-1 in lung-tumors. Peptides 13(1):133–139. doi:10.1016/0196-9781(92)90152-S

Bhutia SK, Maiti TK (2008) Targeting tumors with peptides from natural sources. Trends Biotechnol 26(4):210–217. doi:10.1016/j.tibtech.2008.01.002

Chan SC, Hui L, Chen HM (1998) Enhancement of the cytolytic effect of anti-bacterial cecropin by the microvilli of cancer cells. Anticancer Res 18(6A):4467–4474

Chaudhary J, Munshi M (1995) Scanning electron-microscopic analysis of breast aspirates. Cytopathology 6(3):162–167. doi:10.1111/j.1365-2303.1995.tb00469.x

Chen CX, Hu J, Zeng P, Pan F, Yaseen M, Xu H, Lu JR (2014) Molecular mechanisms of anticancer action and cell selectivity of short alpha-helical peptides. Biomaterials 35(5):1552–1561. doi:10.1016/j.biomaterials.2013.10.082

Conibear AC, Craik DJ (2014) The chemistry and biology of theta defensins. Angewandte Chemie-International Edition 53(40):10612–10623. doi:10.1002/anie.201402167

Craik DJ (2012) Host-defense activities of cyclotides. Toxins 4(2):139–156. doi:10.3390/toxins4020139

Craik DJ, Fairlie DP, Liras S, Price D (2013) The future of peptide-based drugs. Chem Biol Drug Des 81(1):136–147. doi:10.1111/cbdd.12055

de Azevedo RA, Figueiredo CR, Ferreira AK, Matsuo AL, Massaoka MH, Girola N, Auada AV, Farias CF, Pasqualoto KF, Rodrigues CP, Barbuto JA, Levy D, Bydlowski SP, de Sa-Junior PL, Travassos LR, Lebrun I (2015) Mastoparan induces apoptosis in B16F10-Nex2 melanoma cells via the intrinsic mitochondrial pathway and displays antitumor activity in vivo. Peptides 68:113–119. doi:10.1016/j.peptides.2014.09.024

Droin N, Hendra JB, Ducoroy P, Solary E (2009) Human defensins as cancer biomarkers and antitumour molecules. J Proteomics 72(6):918–927. doi:10.1016/j.jprot.2009.01.002

Eliassen LT, Berge G, Leknessund A, Wikman M, Lindin I, Lokke C, Ponthan F, Johnsen JI, Sveinbjornsson B, Kogner P, Flaegstad T, Rekdal O (2006) The antimicrobial peptide, Lactoferricin B, is cytotoxic to neuroblastoma cells in vitro and inhibits xenograft growth in vivo. Int J Cancer 119(3):493–500. doi:10.1002/ijc.21886

Evans J, Wang YD, Shaw KP, Vernon LP (1989) Cellular-responses to pyrularia thionin are mediated by Ca-2$^+$ influx and phospholipase-A2 activation and are inhibited by thionin tyrosine iodination. Proc Natl Acad Sci USA 86(15):5849–5853. doi:10.1073/pnas.86.15.5849

Fang XY, Chen W, Fan JT, Song R, Wang L, Gu YH, Zeng GZ, Shen Y, Wu XF, Tan NH, Xu Q, Sun Y (2013) Plant cyclopeptide RA-V kills human breast cancer cells by inducing mitochondria-mediated apoptosis through blocking PDK1-AKT interaction. Toxicol Appl Pharmacol 267(1):95–103. doi:10.1016/j.taap.2012.12.010

Fernebro J (2011) Fighting bacterial infections-future treatment options. Drug Resist Updates 14 (2):125–139. doi:10.1016/j.drup.2011.02.001

Fosgerau K, Hoffmann T (2015) Peptide therapeutics: current status and future directions. Drug Discov Today 20(1):122–128

Freire JM, Gaspar D, Veiga AS, Castanho MARB (2015) Shifting gear in antimicrobial and anticancer peptides biophysical studies: from vesicles to cells. J Pept Sci 21(3):178–185. doi:10.1002/psc.2741

Furlong SJ, Ridgway ND, Hoskin DW (2008) Modulation of ceramide metabolism in T-leukemia cell lines potentiates apoptosis induced by the cationic antimicrobial peptide bovine lactoferricin. Int J Oncol 32(3):537–544

Galvez AF, Chen N, Macasieb J, de Lumen BO (2001) Chemopreventive property of a soybean peptide (lunasin) that binds to deacetylated histones and inhibits acetylation. Cancer Res 61 (20):7473–7478

Ganz T, Lehrer RI (1998) Antimicrobial peptides of vertebrates. Curr Opin Immunol 10(1):41–44. doi:10.1016/S0952-7915(98)80029-0

Gaspar D, Veiga AS, Sinthuvanich C, Schneider JP, Castanho MARB (2012) Anticancer peptide SVS-1: efficacy precedes membrane neutralization. Biochemistry 51(32):6263–6265. doi:10.1021/bi300836r

Gaspar D, Veiga AS, Castanho MA (2013) From antimicrobial to anticancer peptides. A review. Front Microbiol 4:294. doi:10.3389/fmicb.2013.00294

Gaspar D, Freire JM, Pacheco TR, Barata JT, Castanho MARB (2015) Apoptotic human neutrophil peptide-1 anti-tumor activity revealed by cellular biomechanics. Biochimica Et Biophysica Acta-Molecular Cell Res 1853(2):308–316. doi:10.1016/j.bbamcr.2014.11.006

Gera JF, Lichtenstein A (1991) Human neutrophil peptide defensins induce single-strand DNA breaks in target-cells. Cell Immunol 138(1):108–120. doi:10.1016/0008-8749(91)90136-Y

Guzman-Rodriguez JJ, Ochoa-Zarzosa A, Lopez-Gomez R, Lopez-Meza JE (2015) Plant antimicrobial peptides as potential anticancer agents. Biomed Res Int. doi:10.1155/2015/735087

Harris F, Dennison SR, Singh J, Phoenix DA (2013) On the selectivity and efficacy of defense peptides with respect to cancer cells. Med Res Rev 33(1):190–234. doi:10.1002/med.20252

He WJ, Chan LY, Zeng GZ, Daly NL, Craik DJ, Tan NH (2011) Isolation and characterization of cytotoxic cyclotides from Viola philippica. Peptides 32(8):1719–1723. doi:10.1016/j.peptides.2011.06.016

Henriques ST, Huang YH, Castanho MARB, Bagatolli LA, Sonza S, Tachedjian G, Daly NL, Craik DJ (2012) Phosphatidylethanolamine binding is a conserved feature of cyclotide-membrane interactions. J Biol Chem 287(40):33629–33643. doi:10.1074/jbc.M112.372011

Hernandez-Ledesma B, Hsieh CC, de Lumen BO (2009) Lunasin, a novel seed peptide for cancer prevention. Peptides 30(2):426–430. doi:10.1016/j.peptides.2008.11.002

Holterman DA, Diaz JI, Blackmore PF, Davis JW, Schellhammer PF, Corica A, Semmes OJ, Vlahou A (2006) Overexpression of alpha-defensin is associated with bladder cancer invasiveness. Urol Oncol-Semin Original Inv 24(2):97–108. doi:10.1016/j.urolonc.2005.07.010

Hoskin DW, Ramamoorthy A (2008) Studies on anticancer activities of antimicrobial peptides. Biochimica Et Biophysica Acta-Biomembranes 1778(2):357–375. doi:10.1016/j.bbamem.2007.11.008

Hsieh EA, Chai CM, de Lumen BO, Neese RA, Hellerstein MK (2004) Dynamics of keratinocytes in vivo using (H2O)-H-2 labeling: a sensitive marker of epidermal proliferation state. J Inv Dermatol 123(3):530–536. doi:10.1111/j.0022-202X.2004.23303.x

Huang W, Seo J, Willingham SB, Czyzewski AM, Gonzalgo ML, Weissman IL, Barron AE (2014) Learning from host-defense peptides: cationic, amphipathic peptoids with potent anticancer activity. Plos One 9(2). doi:10.1371/journal.pone.0090397

Hughes P, Dennis E, Whitecross M, Llewellyn D, Gage P (2000) The cytotoxic plant protein, beta-purothionin, forms ion channels in lipid membranes. J Biol Chem 275(2):823–827. doi:10.1074/jbc.275.2.823

Iwasaki T, Ishibashi J, Tanaka H, Sato M, Asaoka A, Taylor D, Yamakawa M (2009) Selective cancer cell cytotoxicity of enantiomeric 9-mer peptides derived from beetle defensins depends on negatively charged phosphatidylserine on the cell surface. Peptides 30(4):660–668. doi:10.1016/j.peptides.2008.12.019

Jenssen H, Andersen JH, Uhlin-Hansen L, Gutteberg TJ, Rekdal O (2004) Anti-HSV activity of lactoferricin analogues is only partly related to their affinity for heparan sulfate. Antiviral Res 61(2):101–109. doi:10.1016/j.antiviral.2003.09.001

Kohno M, Horibe T, Haramoto M, Yano Y, Ohara K, Nakajima O, Matsuzaki K, Kawakami K (2011) A novel hybrid peptide targeting EGFR-expressing cancers. Eur J Cancer 47(5):773–783. doi:10.1016/j.ejca.2010.10.021

Koivunen E, Arap W, Valtanen H, Rainisalo A, Medina OP, Heikkila P, Kantor C, Gahmberg CG, Salo T, Konttinen YT, Sorsa T, Ruoslahti E, Pasqualini R (1999) Tumor targeting with a selective gelatinase inhibitor. Nat Biotechnol 17(8):768–774. doi:10.1038/11703

Kuriyama I, Miyazaki A, Tsuda Y, Yoshida H, Mizushina Y (2013) Inhibitory effect of novel somatostatin peptide analogues on human cancer cell growth based on the selective inhibition of DNA polymerase beta. Bioorg Med Chem 21(2):403–411. doi:10.1016/j.bmc.2012.11.024

Lacerda AF, Vasconcelos EA, Pelegrini PB, Grossi de Sa MF (2014) Antifungal defensins and their role in plant defense. Front Microbiol 5:116. doi:10.3389/fmicb.2014.00116

Leung HW, Wang Z, Yue GGL, Zhao SM, Lee JKM, Fung KP, Leung PC, Lau CBS, Tan NH (2015) Cyclopeptide RA-V inhibits cell adhesion and invasion in both estrogen receptor positive and negative breast cancer cells via PI3 K/AKT and NF-kappa B signaling pathways. Biochimica Et Biophys Acta-Mol Cell Res 1853(8):1827–1840. doi:10.1016/j.bbamcr.2015.04.020

Loeza-Angeles H, Sagrero-Cisneros E, Lara-Zarate L, Villagomez-Gomez E, Lopez-Meza JE, Ochoa-Zarzosa A (2008) Thionin Thi2.1 from Arabidopsis thaliana expressed in endothelial cells shows antibacterial, antifungal and cytotoxic activity. Biotechnol Lett 30(10):1713–1719. doi:10.1007/s10529-008-9756-8

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(4):612–624. doi:10.1158/1535-7163.Mct-04-0077

Mai JC, Mi ZB, Kim SH, Ng B, Robbins PD (2001) A proapoptotic peptide for the treatment of solid tumors. Cancer Res 61(21):7709–7712

Medina SH, Schneider JP (2015) Cancer cell surface induced peptide folding allows intracellular translocation of drug. J Controlled Release 209:317–326. doi:10.1016/j.jconrel.2015.05.267

Mello EO, Ribeiro SFF, Carvalho AO, Santos IS, Da Cunha M, Santa-Catarina C, Gomes VM (2011) Antifungal activity of PvD1 defensin involves plasma membrane permeabilization, inhibition of medium acidification, and induction of ROS in fungi cells. Curr Microbiol 62 (4):1209–1217. doi:10.1007/s00284-010-9847-3

Mohri Y, Mohri T, Wei W, Qi YJ, Martin A, Miki C, Kusunoki M, Ward DG, Johnson PJ (2009) Identification of macrophage migration inhibitory factor and human neutrophil peptides 1-3 as potential biomarkers for gastric cancer. Br J Cancer 101(2):295–302. doi:10.1038/sj.bjc.6605138

Mulder KCL, Lima LA, Miranda VJ, Dias SC, Franco OL (2013) Current scenario of peptide-based drugs: the key roles of cationic antitumor and antiviral peptides. Frontiers Microbiol 4. doi:10.3389/Fmicb.2013.00321

Muller CA, Markovic-Lipkovski J, Klatt T, Gamper J, Schwarz G, Beck H, Deeg M, Kalbacher H, Widmann S, Wessels JT, Becker V, Muller GA, Flad T (2002) Human alpha-defensins

HNPs-1, -2, and -3 in renal cell carcinoma: influences on tumor cell proliferation. Am J Pathol 160(4):1311–1324. doi:10.1016/S0002-9440(10)62558-8

Ngai PHK, Ng TB (2005) Phaseococcin, an antifungal protein with antiproliferative and anti-HIV-1 reverse transcriptase activities from small scarlet runner beans. Biochem Cell Biol-Biochimie Et Biologie Cellulaire 83(2):212–220. doi:10.1139/O05-037

Ortiz-Martinez M, Winkler R, Garcia-Lara S (2014) Preventive and therapeutic potential of peptides from cereals against cancer. J Proteomics 111:165–183. doi:10.1016/j.jprot.2014.03.044

Ouellette AJ, Bevins CL (2001) Paneth cell defensins and innate immunity of the small bowel. Inflamm Bowel Dis 7(1):43–50. doi:10.1097/00054725-200102000-00007

Papo N, Shai Y (2005) Host defense peptides as new weapons in cancer treatment. Cell Mol Life Sci 62(7–8):784–790. doi:10.1007/s00018-005-4560-2

Paredes-Gamero EJ, Casaes-Rodrigues RL, Moura GE, Domingues TM, Buri MV, Ferreira VH, Trindade ES, Moreno-Ortega AJ, Cano-Abad MF, Nader HB, Ferreira AT, Miranda A, Justo GZ, Tersariol IL (2012) Cell-permeable gomesin peptide promotes cell death by intracellular Ca(2$^+$) overload. Mol Pharm 9(9):2686–2697. doi:10.1021/mp300251j

Poon IKH, Baxter AA, Lay FT, Mills GD, Adda CG, Payne JAE, Phan TK, Ryan GF, White JA, Veneer PK, van der Weerden NL, Anderson MA, Kvansakul M, Hulett MD (2014) Phosphoinositide-mediated oligomerization of a defensin induces cell lysis. Elife 3. doi:10.7554/eLife.01808

Reddy KVR, Yedery RD, Aranha C (2004) Antimicrobial peptides: premises and promises. Int J Antimicrob Agents 24(6):536–547. doi:10.1016/j.ijantimicag.2004.09.005

Riedl S, Zweytick D, Lohner K (2011) Membrane-active host defense peptides—challenges and perspectives for the development of novel anticancer drugs. Chem Phys Lipids 164(8):766–781. doi:10.1016/j.chemphyslip.2011.09.004

Rodrigues EG, Dobroff ASS, Cavarsan CF, Paschoalin T, Nimrichter L, Mortara RA, Santos EL, Fazio MA, Miranda A, Daffre S, Travassos LR (2008) Effective topical treatment of subcutaneous murine B16F10-Nex2 melanoma by the antimicrobial peptide gomesin. Neoplasia 10(1):61–68. doi:10.1593/neo.07885

Rosca EV, Koskimaki JE, Rivera CG, Pandey NB, Tamiz AP, Popel AS (2011) Anti-angiogenic peptides for cancer therapeutics. Curr Pharm Biotechnol 12(8):1101–1116

Saar K, Lindgren M, Hansen M, Eiriksdottir E, Jiang Y, Rosenthal-Aizman K, Sassian M, Langel U (2005) Cell-penetrating peptides: a comparative membrane toxicity study. Anal Biochem 345(1):55–65. doi:10.1016/j.ab.2005.07.033

Sah BNP, Vasiljevic T, McKechnie S, Donkor ON (2015) Identification of anticancer peptides from bovine milk proteins and their potential roles in management of cancer: a critical review. Comprehensive Rev. Food Sci. Food Safety 14(2):123–138. doi:10.1111/1541-4337.12126

Salas CE, Badillo-Corona JA, Ramirez-Sotelo G, Oliver-Salvador C (2015) Biologically active and antimicrobial peptides from plants. Biomed Res Int. doi:10.1155/2015/102129

Schroder-Borm H, Bakalova R, Andra J (2005) The NK-lysin derived peptide NK-2 preferentially kills cancer cells with increased surface levels of negatively charged phosphatidylserine. FEBS Lett 579(27):6128–6134. doi:10.1016/j.febslet.2005.09.084

Schweizer F (2009) Cationic amphiphilic peptides with cancer-selective toxicity. Eur J Pharmacol 625(1–3):190–194. doi:10.1016/j.ejphar.2009.08.043

Seo MD, Won HS, Kim JH, Mishig-Ochir T, Lee BJ (2012) Antimicrobial peptides for therapeutic applications: a review. Molecules 17(10):12276–12286. doi:10.3390/molecules171012276

Sharma SV (1992) Melittin resistance - a counterselection for ras transformation. Oncogene 7(2):193–201

Silva ON, Porto WF, Migliolo L, Mandal SM, Gomes DG, Holanda HHS, Silva RSP, Dias SC, Costa MP, Costa CR, Silva MR, Rezende TMB, Franco OL (2012) Cn-AMP1: a new promiscuous peptide with potential for microbial infections treatment. Biopolymers 98(4):322–331. doi:10.1002/bip.22071

Sinthuvanich C, Veiga AS, Gupta K, Gaspar D, Blumenthal R, Schneider JP (2012) Anticancer beta-hairpin peptides: membrane-induced folding triggers activity. J Am Chem Soc 134 (14):6210–6217. doi:10.1021/ja210569f

Stec B (2006) Plant thionins—the structural perspective. Cell Mol Life Sci 63(12):1370–1385. doi:10.1007/s00018-005-5574-5

Svangard E, Burman R, Gunasekera S, Lovborg H, Gullbo J, Goransson U (2007) Mechanism of action of cytotoxic cyclotides: cycloviolacin O2 disrupts lipid membranes. J Nat Prod 70 (4):643–647. doi:10.1021/np070007v

Tanaka T (1997) Chemoprevention of human cancer: biology and therapy. Crit Rev Oncol Hematol 25(3):139–174. doi:10.1016/S1040-8428(97)00232-1

Tanaka T (2009) Colorectal carcinogenesis: review of human and experimental animal studies. J Carcinog 8:5

Torcato IM, Huang YH, Franquelim HG, Gaspar D, Craik DJ, Castanho MARB, Henriques ST (2013a) Design and characterization of novel antimicrobial peptides, R-BP100 and RW-BP100, with activity against Gram-negative and Gram-positive bacteria. Biochimica Et Biophysica Acta-Biomembr 1828(3):944–955. doi:10.1016/j.bbamem.2012.12.002

Torcato IM, Huang YH, Franquelim HG, Gaspar DD, Craik DJ, Castanho MARB, Henriques ST (2013b) The antimicrobial activity of Sub3 is dependent on membrane binding and cell-penetrating ability. ChemBioChem 14(15):2013–2022. doi:10.1002/cbic.201300274

Vesely BA, Eichelbaum EJ, Alli AA, Sun Y, Gower WR, Vesely DL (2006) Urodilatin and four cardiac hormones decrease human renal carcinoma cell numbers. Eur J Clin Invest 36(11):810–819. doi:10.1111/j.1365-2362.2006.01721.x

Walensky LD, Kung AL, Escher I, Malia TJ, Barbuto S, Wright RD, Wagner G, Verdine GL, Korsmeyer SJ (2004) Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. Science 305(5689):1466–1470. doi:10.1126/science.1099191

Wang KR, Yan JX, Zhang BZ, Song JJ, Jia PF, Wang R (2009a) Novel mode of action of polybia-MPI, a novel antimicrobial peptide, in multi-drug resistant leukemic cells. Cancer Lett 278(1):65–72. doi:10.1016/j.canlet.2008.12.027

Wang YS, Li D, Shi HS, Wen YJ, Yang L, Xu N, Chen XC, Chen X, Chen P, Li J, Deng HX, Wang CT, Xie G, Huang S, Mao YQ, Chen LJ, Zhao X, Wei YQ (2009b) Intratumoral expression of mature human neutrophil peptide-1 mediates antitumor immunity in mice. Clin Cancer Res 15(22):6901–6911. doi:10.1158/1078-0432.CCR-09-0484

Wang YS, Li D, Shi HS, Wen YJ, Yang L, Xu N, Chen XC, Chen X, Chen P, Li J, Deng HX, Wang CT, Xie G, Huang S, Mao YQ, Chen LJ, Zhao X, Wei YQ (2009c) Intratumoral expression of mature human neutrophil peptide-1 mediates antitumor immunity in mice. Clin Cancer Res 15(22):6901–6911. doi:10.1158/1078-0432.CCR-09-0484

Wong JH, Ng TB (2005a) Lunatusin, a trypsin-stable antimicrobial peptide from lima beans (Phaseolus lunatus L.). Peptides 26 (11):2086–2092. doi:10.1016/j.peptides.2005.03.004

Wong JH, Ng TB (2005b) Sesquin, a potent defensin-like antimicrobial peptide from ground beans with inhibitory activities toward tumor cells and HIV-1 reverse transcriptase. Peptides 26 (7):1120–1126. doi:10.1016/j.peptides.2005.01.003

Wu DD, Gao YF, Qi YM, Chen LX, Ma YF, Li YZ (2014) Peptide-based cancer therapy: opportunity and challenge. Cancer Lett 351(1):13–22. doi:10.1016/j.canlet.2014.05.002

Yamada Y, Shinohara Y, Kakudo T, Chaki S, Futaki S, Kamiya H, Harashima H (2005) Mitochondrial delivery of mastoparan with transferrin liposornes equipped with a pH-sensitive fusogenic peptide for selective cancer therapy. Int J Pharm 303(1–2):1–7. doi:10.1016/j.ijpharm.2005.06.009

Yang WH, Luo DF, Wang SX, Wang R, Chen R, Liu Y, Zhu T, Ma XY, Liu RH, Xu G, Meng L, Lu YP, Zhou JF, Ma D (2008) TMTP1, a novel tumor-homing peptide specifically targeting metastasis. Clin Cancer Res 14(17):5494–5502. doi:10.1158/1078-0432.CCR-08-0233

Yi ZF, Cho SG, Zhao H, Wu YY, Luo J, Li DL, Yi T, Xu X, Wu ZR, Liu M (2009) A novel peptide from human apolipoprotein(a) inhibits angiogenesis and tumor growth by targeting c-Src phosphorylation in VEGF-induced human umbilical endothelial cells. Int J Cancer 124 (4):843–852. doi:10.1002/ijc.24027

Zhao BX, Zhao Y, Huang Y, Luo LM, Song P, Wang X, Chen S, Yu KF, Zhang X, Zhang Q (2012) The efficiency of tumor-specific pH-responsive peptide-modified polymeric micelles containing paclitaxel. Biomaterials 33(8):2508–2520. doi:10.1016/j.biomaterials.2011.11.078

Zheng LH, Wang YJ, Sheng J, Wang F, Zheng Y, Lin XK, Sun M (2011) Antitumor peptides from marine organisms. Marine Drugs 9(10):1840–1859. doi:10.3390/md9101840