A REVIEW OF POTENTIAL ANTICANCERS FROM ANTIMICROBIAL PEPTIDES

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ABSTRACT

Cancer is one of the leading causes of morbidity and mortality globally. The drawbacks of conventional chemotherapy such as resistance, lack of specificity, severe toxicity warrant the need to explore alternative approaches for the treatment of cancer. Antimicrobial peptides are part of the innate defense mechanism of all organisms and have been developed as potential alternatives in combating infectious diseases. In addition, antitumor effects of many peptides have been reported with remarkable prospects in some in vitro studies especially on breast, cervical and lung cancer cell lines, and in vivo murine tumour xenografts. This review summarizes the reports on the activities of some selected antitumor peptides on various cancer cell lines.

Keywords: Antimicrobial peptides, Anticancer/antitumour peptides, Host defense peptides.

INTRODUCTION

Antimicrobial peptides (AMPs) are diverse, ancient and evolutionary conserved components of the innate immune system present in all forms of life [1] and have been isolated from various species (table 1). AMPs are also termed as host defense peptides. They are cationic and amphipathic in nature; this property played a facilitatory role in their binding and insertion into the anionic cell membrane of microorganisms. AMPs possess low propensity for developing resistance, probably due to their distinguished mode of action [2]. Consequently, they show broad spectrum antimicrobial activities against various micro-organisms, including Gram-positive and Gram-negative bacteria, fungi and viruses [3]. Over 2000 AMPs, mostly cationic, have been reported from the antimicrobial peptide database [4] and the number keep increasing with newer research discovery. In addition to their antimicrobial function, AMPs also exert antitumor effect, immunomodulatory effect, and wound-healing effect. They also find applications as drug delivery vector, contraceptive agent, mitogenic agent and signal molecules in signal transduction pathways [5].

Owing to the diversity of functions shown by the antimicrobial peptides, they can also be categorized into antibacterial peptides, antiviral peptides, antifungal peptides, antiparasitic peptides, anticancer/antitumour peptides, anti-HIV peptides, anti-protist peptides, insecticidal peptides, spermicidal peptides and AMPs with chemotactic activity [1]. However, one AMP may have or fall into more than one group. Although, AMPs have a certain degree of similarity among themselves regarding the biophysical properties, their sequence is rarely similar among closely related or distinct species [6].

Classification of AMPs

Host defense peptides can majorly be classified based on their secondary structure into four different groups or families which are: i) alpha (α), ii) beta (β), iii) alpha beta (αβ) and non-alpha beta (non-αβ).

The α-family consists of AMPs with helical structure, e.g. magainin-II (Fig. 1E). The β-family is composed of AMPs with beta strands; e.g. looped thanatin, C = β-sheeted polyphemusin, D = rabbit kidney defensin-1, E = α-helical magainin-2, F = extended indolicidin.

Mode of action of antimicrobial peptides

Antimicrobial peptides have been termed “natural antibiotics” due to broad spectrum activity against micro-organisms [8]. Cationic peptides interact directly with the negatively charged cellular membrane of bacteria cells resulting in enhanced membrane permeability and ultimately leading to swift cell lysis. [29]. So in effect the peptide can cause membrane disruption or membrane interaction, which may lead to the formation of transient pores and transport of the peptide into the cell thereby ensuring contact with the intracellular targets [30]. The four commonly used models to describe the mechanism of AMP action include: toroidal, carpet-like, barrel-stave and aggregate channel models.
Table 1: Diverse sources of antimicrobial peptides

<table>
<thead>
<tr>
<th>Source</th>
<th>Scientific name</th>
<th>Host defense peptides</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Homo sapien</td>
<td>Dermicidin (anionic)</td>
<td>[7]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LL-37</td>
<td>[7]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HNP-1</td>
<td>[7]</td>
</tr>
<tr>
<td>Insects</td>
<td>Hyalophora cecropia (Silk moth)</td>
<td>Cecropin</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td>Apis mellifera (honey bee)</td>
<td>Melittin</td>
<td>[9]</td>
</tr>
<tr>
<td></td>
<td>Drosophila melanogaster (fruit fly)</td>
<td>Drosomycin</td>
<td>[10]</td>
</tr>
<tr>
<td>Plants</td>
<td>Leonurus cardiac (Motherwort)</td>
<td>Lipid transfer protein</td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td>Viscum album L.</td>
<td>Viscotoxin A1</td>
<td>[13]</td>
</tr>
<tr>
<td></td>
<td>Benincasa hispida</td>
<td>Hispidalin</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td>Viscum album L.</td>
<td>Hepcidin TH1-5</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>Chionodraco hamatus (Marine icefish)</td>
<td>Chionodrachine</td>
<td>[16]</td>
</tr>
<tr>
<td>Fishes</td>
<td>Oreochromis mossambicus (Freshwater tilapia)</td>
<td>Crotamine</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>Chionodraco hamatus (Marine icefish)</td>
<td>Crotamine</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>Bungarus fasciatus (Branded krait)</td>
<td>BF-CATH</td>
<td>[20]</td>
</tr>
<tr>
<td></td>
<td>Emys orbicularis (European pond turtle)</td>
<td>TBD-1</td>
<td>[21]</td>
</tr>
<tr>
<td>Amphibians</td>
<td>Bufo bufo gargarizans (Asian toad)</td>
<td>Buforin-I</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td>Xenopus laevis (African clawed frog)</td>
<td>Magainin-II</td>
<td>[18]</td>
</tr>
<tr>
<td>Reptiles</td>
<td>Crotalus durissus terrificus (American rattlesnake)</td>
<td>Crotamine</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>Bungarus fasciatus (Branded krait)</td>
<td>BF-CATH</td>
<td>[20]</td>
</tr>
<tr>
<td></td>
<td>Emys orbicularis (European pond turtle)</td>
<td>TBD-1</td>
<td>[21]</td>
</tr>
<tr>
<td>Birds</td>
<td>Gallus gallus (chicken)</td>
<td>Fowlicidin-1</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>Penguin</td>
<td>AvBD 103a</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>Anas platyrhynchos (mallard duck)</td>
<td>AvBD 103a</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>Rhinoglossus microsorus (fungus)</td>
<td>Rhininsin-1</td>
<td>[23]</td>
</tr>
<tr>
<td>Microbe</td>
<td>Pseudoplectania nigrilla (fungus)</td>
<td>NZ2114</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>Lactococcus lactis (bacterium)</td>
<td>Nisin</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>Streptococcus pneumoniae (bacterium)</td>
<td>Pep27 anal2</td>
<td>[26]</td>
</tr>
</tbody>
</table>

HNP-1 human neutrophil peptide I, AvBD avian β-defensin, BF-CATH B. fungarus cathelicidin, TBD-1 turle β-defensin 1, NZ2114 plectasin

Table 2: Antimicrobial peptides based on their structural features

<table>
<thead>
<tr>
<th>AMP class</th>
<th>Structural features</th>
<th>Typical examples</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cationic peptides</td>
<td>Peptide forming helical structures</td>
<td>Cepharins</td>
<td>α-helix</td>
</tr>
<tr>
<td></td>
<td>One disulphide bridge</td>
<td>Thanatin</td>
<td>β-helix</td>
</tr>
<tr>
<td></td>
<td>Two disulphide bridge</td>
<td>Tachyplesins II</td>
<td>β-helix</td>
</tr>
<tr>
<td></td>
<td>Three disulphide bridge</td>
<td>Panaeidines</td>
<td>β-helix</td>
</tr>
<tr>
<td></td>
<td>Greater than three disulphide bridges</td>
<td>Drosomycin</td>
<td>αβ structure</td>
</tr>
<tr>
<td></td>
<td>Glycine-rich peptides</td>
<td>Pyrrhocorcin</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Histidine-rich peptide</td>
<td>Dipterisin</td>
<td>Rich in H</td>
</tr>
<tr>
<td></td>
<td>Proline-rich peptide</td>
<td>Histatin</td>
<td>αβ structure</td>
</tr>
<tr>
<td></td>
<td>Tryptophan –rich peptide</td>
<td>Indollicid</td>
<td>Extended structure</td>
</tr>
<tr>
<td>Non-ionic peptides</td>
<td>Aspartic acid-rich peptide</td>
<td>Dermicidin</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Neuropeptide-derived molecules</td>
<td>Secretolycin</td>
<td>α-helix</td>
</tr>
<tr>
<td></td>
<td>Oxygen binding proteins</td>
<td>Lactoferricin</td>
<td>β-turn</td>
</tr>
<tr>
<td></td>
<td>Aromatic dipeptides</td>
<td>N-α-lysyl-5-s-glutathionyl</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-3,4-dihydroxyphenylalanine</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&amp; P-hydroxy cinnamaldehyde</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from Pushpanathan et al. [4] N/A = not available

In toroidal model, AMPs align perpendicularly into the bilayer structure with their hydrophobic regions associated with the central part of the lipid bilayer and their hydrophilic regions facing the pore [31]. Whereas, in carpet-like mode (also known as detergent-like test), the peptide micelle touches the membrane first and coats a small area of the membrane. Thereafter, AMP penetrate the lipid layer to allow pore formation occur leaving holes behind [32]. In the barrel-stave model, staves are formed first parallel to the cell
membrane. Then barrels are formed and AMPs are inserted perpendicularly to the plane of the membrane bilayer [33]. On the other hand, AMPs glue themselves to the membrane parallel to the surface in an aggregate channel model. Then reorientation of AMPs occur and they insert themselves into the membrane vertically to form sphere-like structures [32]. The schematic diagram of the models is illustrated in fig. 2.

Fig. 2: Illustration of the models of lipid membrane permeabilization by antimicrobial peptides. Adapted from Silva et al. [34]

**AMPs as anticancer therapeutics**

Cancer is the most malignant disease threatening the health of man. It has been predicted that the number of death from this disease will continue to increase with an estimated 11.5 million deaths by the year 2030 [35]. Conventional chemotherapeutics has many drawbacks such as resistance; lack of selectivity, severe toxicity hence the need to explore alternative approach for cancer remission. AMPs have emerged as potentially better alternative as they have broad anticancer spectrum with low propensity for resistance, selectivity for cancer cells, rapid cell kill, ability to destroy and prevent metastasis of primary tumours and are harmless to vital organs [36]. This review highlights some of the antimicrobial peptides that have been reported to possess anticancer properties.

**Anticancer mechanism of AMPs**

Three anticancer mechanisms have been proposed for cytotoxic AMPs namely: a) cell membrane lysis; b) inhibition of angiogenesis; and c) activation of extrinsic apoptotic pathways [8].

**Cell membrane lysis**

This mechanism is similar to the mode of action of antimicrobial peptides that disrupt the bacterial cell wall and the effect may involve selective lysis of the cancer cell membrane. Anticancer peptides (ACPs) memranolytic and selective mode of action on tumor cells can be due to the increased anionicity of the cytoplasmic membrane of these cells [37]. This follows the same pattern as the “carpet” and “barrel-stave” models. According to the carpet mechanism [38], the peptides align themselves parallel to the negatively charged membrane surfaces in a carpet-like fashion until a critical threshold concentration is attained, after which the peptide permeates the membrane. Conversely, for “barrel-stave” model, the lytic cationic amphiphilic peptides self-aggregate via hydrophobic interactions in the membrane and form transmembrane channels or pores [39]. Other membraneolytic activities involve the permeation and swelling of mitochondria with release of cytochrome c and apoptosis events [40]. Examples of some oncolytic peptides are listed in table 3.

**Inhibition of tumour angiogenesis**

Angiogenesis defined as the process of new blood vessel formation is essential during cancer progression [41]. During this process, migration and proliferation and entry into tumour surrounding tissue, of endothelial cells occur forming a capillary network [42]. It plays an essential role in the growth, invasion and metastasis of solid tumours, which could provide necessary nutrients and oxygen and discard metabolic wastes [43]. Present in the tumour cells are angiogenic growth factors that stimulate the process of angiogenesis. These include vascular endothelial growth factor (VEGF-A), fibroblast growth factor, epidermal growth factor, platelet-derived growth factor, placental growth factor, angio poietin and other activators [44]. Many peptides could unleash beneficial angiogenesis and angioteurm effects mainly by interrupting the interactions between growth factors and their receptors [44]. Attempts at controlling tumourigenesis involve targeting the angiogenesis processes [45].

**Activation of extrinsic apoptotic pathway**

Multicellular organisms have developed apoptosis to control cell metastasis in response to DNA damage, during cell development and cellular stress [46]. In the course of apoptosis, the nucleus and cytoplasm shrink, condense and then fragment, releasing small membrane-bound apoptotic bodies, which are phagocytosed by macrophages or adjacent cells [47]. The extrinsic apoptotic pathway is receptor-mediated and regulated by tumour necrosis factor (TNF) super family namely Fas (CD95) and TNF-related apoptosis-inducing ligand (TRAIL) receptors. Binding of these receptors with their ligands such as Fas ligand (Fasl) and TRAIL respectively activate this pathway [48]. Increasing evidence suggest that apoptosis could play a significant part in getting rid of cancer cells without eliciting damage to the healthy normal cells or surrounding tissues [49]. Targeting apoptosis pathways in premalignant and malignant cells will be effective strategies for cancer prevention and treatment [50]. Conventional chemotherapeutic drugs may induce apoptosis by enhancing Fas expression [48]. Peptides that could induce apoptosis in tumor cells are slowly becoming important candidates for the development of new anticancer drugs [44]. The mode of action for anticancer peptides may not be limited to those highlighted above. It is not uncommon, however, to find a peptide with multiple mechanisms in exerting their effect [37].

A12L/A20L & Pep27anal2

Huang et al. [66] demonstrated the role of helicity on the anticancer mechanism of action of cationic helical peptides. The 26-residue amphiphatic α-helical peptide A12L/A20L was modified by replacing the original L-amino acid with the D-form on either polar or non-polar face of the helix. This results in improved therapeutic index of the peptide against human cervix adenocarcinoma cells (HeLa) by 9-fold and 22-fold respectively. The anticancer activity of these peptides can also be correlated to their hydrophobicity. When challenged with eight different cell lines including HeLa, human breast adenocarcinoma cell line (MCF7), human lung carcinoma cell line (A549), mouse melanoma cell line (B16), the peptides showed greater anticancer activity against cervical carcinoma cells with IC50 of 2 and 1.2 μmol/l with a necrotic-like membrane disruption mechanism. Thus increasing hydrophobicity can lead to increased anticancer effect [67]. Similar phenomenon stem from introducing tryptophan in the peptide pep27 analogues with increased anticancer activity shown by Pepanal2 against acute myelogenous leukaemia cell line (AML-2), acute promyelocytic leukaemia cell line (HL60), gastric cancer cell line (SNU-601), Jurkat T cell leukaemia cell line and MCF7 [26].

Cecropin A & B

Cecropin A and B are members of cecropin-family of AMP first derived from the giant silk moth, Hyalophora cecropia. These peptides exerted selective inhibitory and antiproliferative efficacy against bladder tumour cell lines namely RT4, 647V, J82, 486P in a dose-dependent fashion. However, both peptides remained benign to fibroblasts of murine and human origin [68]. In a related research, Wu et al. [69] reported that another derivative of cecropin, synthetic peptide CBL1a, showed selective anticancer activity against leukemia, stomach carcinoma and lung cancer cells but low toxicity against non-cancer cells. The IC50 of this potential anticancer agent in leukemia and stomach carcinoma were 2-8 folds.
lower than the parent peptide cecropin. CB1a also displayed high selective toxicity against two lung cancer cells – NCI-H460 and NCI-H520. The peptide had an interaction time with the target cell (IT₅₀) of 7 mins, which suggest that the drug was fast acting. More extensive study was done on this peptide where it, at 5mg/kg, inhibited the growth of lung tumours in an in vivo mouse model xenografted with human tumorigenic NCI-H460 lung cancer cells [64]. Comparatively, the peptide was toxic to cancerous cells and less toxic to normal cells than conventional antitumour drug, docetaxel. The peptide can survive long enough in the bloodstream to exert its effect before protease digests it; this longevity is due to its design of three repeated amphipathic sequence [64].

<table>
<thead>
<tr>
<th>Peptide name</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aurein 1.2</td>
<td>GLFDKIKIAEFS</td>
<td>[51]</td>
</tr>
<tr>
<td>Hepcidin TH1-5</td>
<td>GIKCRFCGGCGCTGIGVCRCRF</td>
<td>[52]</td>
</tr>
<tr>
<td>Melittin</td>
<td>GIGAVLKWTTGLPAISWIKRRQQ</td>
<td>[53]</td>
</tr>
<tr>
<td>Epinecide-1</td>
<td>GFPIHFLKHFHMKIMHGLV</td>
<td>[54]</td>
</tr>
<tr>
<td>Tachyplesin</td>
<td>LGCFRRLSPKCRGCMRCRCCR</td>
<td>[55]</td>
</tr>
<tr>
<td>LL-37</td>
<td>LLGDFRKRSEKIGKEFRRVORHDGRFLNLPRTES</td>
<td>[43]</td>
</tr>
<tr>
<td>Cercopin (CB1a)</td>
<td>KWKVFHKIEK-KWKVFHKIEK-APKWVFHKIEK</td>
<td>[56]</td>
</tr>
<tr>
<td>Lactofercin</td>
<td>FKCRRWQWRMMLKGLAPSTCVRRAF</td>
<td>[57]</td>
</tr>
<tr>
<td>TαAP-1/TαAP-2</td>
<td>FSLIPSVGGISASAFK-FGMPGLGLGSASAFK</td>
<td>[58]</td>
</tr>
<tr>
<td>Baforin-II</td>
<td>TRSSARQLQFQPRVRHRLRRK</td>
<td>[59]</td>
</tr>
<tr>
<td>Magainin-II</td>
<td>GIGKFLEASKIFGKAFVGKEMINS</td>
<td>[60]</td>
</tr>
<tr>
<td>Heparin TH2-3</td>
<td>QSLHSLRCWRCNCRCSNCGCS</td>
<td>[61]</td>
</tr>
<tr>
<td>Gomesin</td>
<td>ZCRRLCRKQCVQTYCRGR</td>
<td>[62]</td>
</tr>
<tr>
<td>Temporin-1CEa</td>
<td>PVDDKIKANININSFKG</td>
<td>[63]</td>
</tr>
<tr>
<td>Pardaxin</td>
<td>GFFALPRISPSPLFRTLSSAVGLSSGGQE</td>
<td>[64]</td>
</tr>
<tr>
<td>Piscidin-1</td>
<td>FFSHPGR1VHGVTTHHLYTG</td>
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</tr>
<tr>
<td>HNP-1</td>
<td>AYCRYPACAIGEYRTGYQGIRLWFFCC</td>
<td>[66]</td>
</tr>
<tr>
<td>GW-H1</td>
<td>GYNYKKLANKKAFANALW</td>
<td>[67]</td>
</tr>
<tr>
<td>Pep27anal2</td>
<td>MWKWFHNLSWLLWLADKPRAPDRNK</td>
<td>[68]</td>
</tr>
<tr>
<td>Cercopin A</td>
<td>KWKFLKFKIEKGGNQRDRGKIAKAPAVAVQGTQIAK</td>
<td>[69]</td>
</tr>
<tr>
<td>Cercopin B</td>
<td>KWKVFHKIEKGMGNIRGNYKAGPAVLGEAKAL</td>
<td>[70]</td>
</tr>
</tbody>
</table>

**Magainin-II**

Magainin-II belongs to the magainins AMP family, and the parent peptide was initially isolated from the skin of African clawed frog, *Xenopus laevis* [3]. Magainins and its derivatives have been shown to exert anticancer effect on a number of cancer cells. Significant cytotoxicity of magainin-II have been reported against cancer cells including bladder tumours [70], HeLa cells [71], human melanoma [72], lung cancer cells [73]. In vivo, magainins have been shown to improve the recovery of animals with cancer-producing tumours [74]. In addition, nude mice xenografted with melanoma tumour were able to recuperate completely after local treatment with magainin-II. Selectivity for neoplastic cells over normal cells is one of the main positives of magainin peptides [70], they are also reported to resist degradation by proteases [75].

**Melittin**

Melittin is a 26 amino acid sequence linear peptide that is the principal component of bee venom. It is a potent activator of phospholipase A2, caspase and matrix metalloproteinase that kill tumour cells [76]. It has been reported as a lytic peptide with broad spectrum of anticancer effects, but its toxicity towards normal cells has limited its potentials as a therapeutic agent [9]. However, this drawback can be subjugated by introducing proper delivery vehicle in the form of melittin nanoparticles that can safely convey the peptide in significant amount intravenously to target and destroy the cells [9]. Other AMP and their analogues derived from bees have been shown to be cytotoxic especially to HeLaS3, a clonal derivative of parent HeLa cell line [77].

**LL-37/hCAP-18**

Human cationic AMP of 18 kDa is the human cathelicidin-derived peptide initially synthesized as a preprotein (hCAP-18) before being subsequently converted into its active form LL-37 of 4.5 kDa [78]. A COOH moiety of hCAP-18 consisting amino acid residue 109-135 (hCAP109-135) and which correspond to LL-37 in vivo, residue induce apoptosis in human oral squamous carcinoma cell line by mitochondrial depolarization without any detectable activation of caspase-3 [79]. This finding support the proposition that the oncolytic activity of LL-37/hCAP-18 is due to LL-37 (17-29), which is a 13-amino acid fragment attached to COOH-terminal region corresponding to amino acid residue 17-29 [80].

Although LL-37 (17-29) is equally cytotoxic to drug-sensitive and drug-resistant variants of the KB human squamous cancer cell line, it also kills untransformed human endothelial cells. Native LL-37 is similarly cytotoxic to human peripheral blood lymphocytes [81]. This toxicity against normal human cells has limited their therapeutic potentials.

**Pardaxin**

Pardaxin is a 33-amino acid sequence amphipathic polypeptide neurotoxin originally extracted from the marine fish *Pardachirus marmoratus*. Few studies have reported the anticancer potential of this AMP. Wu et al. [82] reported that pardaxin exhibited antitumour activity against murine fibrosarcoma both in vivo and in vitro. The synthetic peptide, at 25mg/kg, 0.5mg/day inhibit the proliferation of MN-11 cells after 14 d and reduce colony formation in a soft agar assay. By caspase-dependent and reactive oxygen species (ROS)-dependent apoptotic mechanism, the peptide inhibited human fibrosarcoma cells (HT1080) proliferation [83]. Pardaxin induced programmed cell death in HeLa cells but inhibited proliferation of HT1080 cells via a different mechanism [84].

**Baforin-IIB & Aurein 1.2**

Baforin-IIB is a 21-amino acid peptide derivative of baforin I (a 39-amino acid peptide first isolated from the Asian toad *Bufo bufo gargarizanum*). Both peptides comprise helix-hinged-helix structures derived from histone H2A [85]. Baforin IIB exerts a broad spectrum of anticancer activity; the peptide have been reported to exert cytotoxicity on a remarkably 62 cancer cell lines by explicitly targeting cancer cells through interaction with cell surface gangliosides. It stimulates apoptosis by mitochondria-dependent pathway as authenticated by caspase-9 activation and cytochrome c release to cytosol besides DNA laddering and annexin V-FITC double staining [86]. Aurein 1.2, another peptide of amuran origin showed anticancer effect in 50 out of 60 cancer cell lines tested against by National Cancer Institute (NCI) tumour line testing program [87].
NRC-03 & NRC-07

 Pleurocidin-family cationic AMPs – NRC-03 & NRC-07 were shown to be cytotoxic to multiple breast cancer cells including MCF7, TX400 cells that overexpress p-glycoprotein and slow-growing breast adenocarcinoma (SKBR3) cells by binding to the negatively charged molecules on the cancer cells [88]. NRC-03 substantially reduces the median effective concentration EC50 of cisplatin for breast cells implying its use as a chemosensitizer. Both peptides killed breast cancer cells grown as xenografts in non-obese diabetic, severely combined immune-deficient (NOD SCID) mice strains [88].

AMP containing the CitobDGRC (CDK4) motif displayed cytotoxicity in aminopeptidase (CD13) negative breast cancer cells MCF7 & MDA-MB-231 in vitro. The peptide reduced mitochondrial membrane potential, promoted caspase-3 and inhibited Bcl-2 expression in the breast cancer cell lines. Moreover, the peptide inhibited the progression of the xenograft tumour and the generation of neovascularization in nude mice in vivo [89].

Temporin-1Ca

Temporin-1Ca is a 17-residue amphiphilic α-helical AMP isolated from the skin secretion of the Chinese brown frog *Rana chensinensis* and has been reported to exert rapid cytotoxicity against human breast cancer cell lines [90]. The anticancer mechanisms of the peptide against two human breast cancer cell lines, MCF7 and MDA-MB-23, have been investigated. Temporin-1Ca triggers rapid cell death in breast cancer cells by intracellular Ca²⁺ leakage, over-generation of reactive oxygen species (ROS), collapse of mitochondrial membrane potential [90]. Further study of temporin-1Ca on Eκα-negative human breast cancer cell line Bcap-37 yielded the same cytotoxic mechanisms in line with previous report [61].

TH1-5 & TH2-3

Hepcidins are cysteine-rich peptides that have been identified in many vertebrates including humans, amphibians, reptiles and fish [91]. Fish hepcidin have been shown to exert cytotoxicity activity against cancer cells. Tilapia hepcidin TH2-3, a synthetic 20-mer AMP from *Oreochromis mossambicus*, inhibited the growth and migration of HT1080 in a concentration-dependent manner. The peptide also caused lethal membrane disruption in HT1080 cancer cells. Real-time PCR and migration assay suggest that the peptide possesses cytolytic activity and downregulates the e-Jun gene in cancer cells [92]. TH1-5, another peptide from *O. mossambicus* inhibited the proliferation of HeLa, HepG2 (human hepatocellular carcinoma), and HT1080 tumour cells by altering membrane disruption and inducing apoptosis at low dose. In addition, TH1-5 also expressed modulation of immune-related genes [52].

Epinecandin-1 and Piscicidin-1

Epinecandin-1 is a synthetic 21-mer peptide originally gotten from the grouper *Epinephelus cooides*. It displayed cytotoxic activity against several cancerous cell lines including A549, HT1080, HepG2, HeLa, human kidney cell (WS-1), mouse fibroblast cell (H1N3T3), murine hepatocyte cell (AML-12), human hepatic tumor-derived cell (HASSFT/VGH) and mouse macrophage from Abelson murine leukemia virus-induced tumour (RAW264.7) [54]. Epinecandin-1 increased the cytotoxicity of these cancer lines in a dose and time-dependent manner, which suggest that cell death occurred by membrane disruption [54]. Again it inhibited the proliferation of human leukemia cells U937 and induce apoptosis in response to cytokine reproduction [93]. Piscicidin-1 is a 22-residue peptide with a cationic α-helical structure isolated from the mast cells of hybrid striped bass *Morone saxatilis* and *M. chrysops* [94]. Lin et al. showed that Piscicidin-1 at low doses induce both apoptosis and necrosis in HT1080 cells. It also triggers a necrotic cell death pathway in a short period of high-dose treatment. In addition, piscicidin-1 also inhibits the migration of HT1080 cells in a dose-dependent manner [94].

A.K & GW-H1

Resembling conventional surfactants, A.K is a short designed amphiphilic peptide that comprises a hydrophilic lysine residue as the C-terminus, followed by nine consecutive hydrophobic alanine residues [95]. The peptide exhibited high selectivity and dual modes of antitumour effects on the cancerous cells by membrane cell disruption and induction of cell apoptosis. It inhibited the growth of HeLa and human promyelocytic leukemia cells (HL60). One unique feature of this synthetic peptide is that it is not degraded by protease [95]. GW-H1 is a synthetic cationic amphiphilic AMP with α-helix conformation. It inhibited the viability of three hepatocellular carcinoma cell line (J5, Huh7 and Hep3B) [64]. GW-H1 exerts high selective cytotoxicity against J5 cell line via caspase-dependent apoptosis. Also, in vivo it inhibited the growth of J5 xenografts in nude mice suggesting its potential antitumour capacity [64].

TsAP-1 & TsAP-2

TsAP-1 and TsAP-2 are two novel peptides obtained from the venom of the yellow Brazilian scorpion *Tityus serrulatus*. Both peptides exhibit 17-mer amidated linear and non-disulfide bridged peptides. [58]. Lysine residue substitution of these two peptides enhanced their potency against five human cancer cell lines namely human squamous adenocarcinoma cell line (NCI-H57), human lung adenocarcinoma cell line (NCI-H83), human androgen-independent prostate adenocarcinoma cell line (PC-3), human glioblastoma cell line (U251) and MCF7. TsAP-2 is more potent of the two; has IC50 values ranging between 0.83 and 2.0µM [58].

Lactoferricin

Lactoferricin is a 25-amino acid AMP obtained by acid-pepsin hydrolysis of mammalian lactoferrin [57]. The amphibatic cationic peptide has been reported to be cytotoxic to murine and human cancer cell lines including leukemia, fibrosarcoma, various carcinomas and neuroblastoma cells [96–99]; this cytotoxicity occurs at concentrations that do not significantly affect normal cells such as normal fibroblasts, lymphocytes, epithelial cells, endothelial cells or erythrocytes [98,100]. In vivo, LfcinB also exerts potent antitumour effect on mice inoculated with LS178-ML25 murine lymphoma and B16-BL6 murine melanoma cells [96]. Limitations of this peptide as a potential anticancer therapeutic is its susceptibility to enzymatic digestion as well as inactivation by anionic serum components.

Gomesin

Gomesin is a potent 18-amino residue AMP isolated from haemocytes of the spider *Acanthoscurria gomesiana*; its anticancer activity both in vitro and in vivo has been tested [60]. Gomesin exerted direct cytotoxicity on murine and human tumour cells in vitro with an estimated IC50 for the murine melanoma B16F10-Nex2 being 3.5µM and <10 µM for the human tumour cell lines (HeLa, SKBR3, LS180). In vivo, local treatment with gomesin-containing cream significantly increased the survival time of murine melanoma-challenged mice as shown by delayed growth [60].

BMAP-27 & BMAP-28

Bovine myeloid antimicrobial peptides (BMAP) are α-helical cationic peptides of the cathelicidin family. Despite their narrow therapeutic range, few studies have evidenced that they possess cytotoxic activity on certain human leukemic cells by apoptotic induction [101–103]. At high doses, they cause the hemolysis of benign erythrocytes and leukocytes [101,103].

Tachyplesin

Isolated from the haemocytes of the horseshoe crab *Tachypleus tridentatus*, the 17-amino acid peptide is characterized by two antiparallel β-sheets rigidly held in place by two disulphide bonds. Its antitumour mechanism involves a multistep model in which the peptide binds to hyaluronan on the surfaces of target cells and forms a membrane attack complex (MAC) at the final stage. This complex consequently disrupts the plasma membrane integrity and thereby kill the target cells [55]. Preferential expression of hyaluronan on the surface of malignant and endothelial cells involved in cancer vascularization suggests that the peptide inhibit the proliferation of cells relative to other non-tumorogenic cell lines that express less of hyaluronan on their surfaces [104]. Tachyplesin has been shown to inhibit malignant growth in the presence of normal serum even against cells that over express the multiple-drug resistant gene [55]. A synthetic version of tachyplesin conjugated to the integrin homing domain RGD blocked the growth of tumor cells both in vitro and in vivo.
vivo [104]. RGD-tachyplesin has been shown to inhibit the proliferation of both cultured malignant and endothelial cells and reduced the colony formation of TSU prostate cancer cells. In vivo, it has been reported to inhibit the growth of neoplasms via induction of apoptosis in both malignant and endothelial cells evidenced by activation of several caspases in both the mitochondrial and Fas-dependent pathways [55].

CONCLUSION

Anticancer antimicrobial peptides hold promise to yielding novel drugs for the treatment of cancer that could replace the traditional neoplastic agents or used in combination therapy. Several studies have documented the remarkable activities of these molecules in several cancers; this is evident in the number of the ACPs in the database and documented literature. Nevertheless, further studies into this emerging field of anticancer peptide need to be embraced. More into this anticancer need to be embraced. More in vitro studies need to be conducted correlating with the effects reported on numerous cancer cell lines. Understanding the detailed and precise mechanisms of this class of agents and structure-activity relationship will provide a knowledge platform for answering some of unanswered questions about AMPs and designing superior peptides. Instability and proteolytic degradation of the peptides need to be further studied as bypassing this ‘hostility’ allow these agents to exert their full therapeutic potentials and perhaps decipher some activities yet unknown.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES


