REVIEW PAPER

OVERVIEW OF HOST DEFENSE PEPTIDES WITH PROMISING ANTI-BREAST CANCER ACTIVITY

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Received: 30.06.2021.
Accepted: 27.07.2021.

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DOI: 10.2478/sjecr-2021-0052

ABSTRACT

Breast cancer is the leading cause of death among women worldwide. The main limitations of conventional anti-cancer therapy, including breast cancer treatment, are side effects and the development of resistance to chemotherapeutics. Host defense peptides (HDPs) are bioactive compounds of innate immunity isolated from almost all living organisms, which exhibit wide range of biological activities. This review focuses on the anti-cancer effects of HDPs and their therapeutic potential against breast cancer. Numerous HDPs from different sources, including mammalian and amphibian origin, and their chemically modified analogues, exert the spectrum of anti-cancer activities. These effects include direct disruption of cancer cell membrane, induction of apoptosis, inhibition of angiogenesis and cancer cell proliferation, but also the modulation of anti-cancer immune response. Selected examples of HDPs of different origin and their anti-breast cancer capacities have been reviewed. Conclusively, due to their anti-cancer effects accompanied by substantial selectivity for cancer cells and low toxicity for normal cells, HDPs have been widely recognized as possible therapeutic agents.

Keywords: *Breast cancer, host defense peptides, anti-cancer activity, cytotoxicity, therapy.*

ABBREVIATIONS

HDP - host defense peptide
NK cells - natural killer cells
HNPs - human neutrophil peptides
HER2 - human epidermal growth factor receptor 2
hBD1 - human beta-defensine-1
bLf - bovine lactoferrin
mTOR - mammalian target of rapamycin
hLf - human lactoferrin
LfcinB - lactoferricin B
CAMP - cathelicidin antimicrobial peptide
PI3K - phosphatidylinositol-3-kinase
Akt - PKB – serine/threonine protein kinase B
TAMs - tumor associated macrophages
ROS - reactive oxygen species
P-gP - P glycoprotein
IL - interleukin
TNF-α - tumor necrosis factor-α
TGF-β - transforming growth factor-β
NF-κB - nuclear factor-κB
IFN-γ - interferon-γ

INTRODUCTION

Breast cancer is the most common cancer and the leading cause of death among women worldwide. In 2020, approximately 2.2 million of newly diagnosed breast cancer cases and ~685 000 deaths were reported (1). Like other cancers, breast cancer is a highly heterogeneous disease that is driven by genetic and epigenetic modifications. In addition, the ambient in tumor microenvironment may further contribute to breast cancer heterogeneity (2). Considering the fact that breast cancer is heterogeneous tumor, its treatment is complex and multidisciplinary. The conventional therapy for breast cancer, applied separately or in combination, includes surgical resection and radiation therapy, chemotherapy, as well as immunotherapy based on the monoclonal antibody and checkpoint inhibitors application (3). However, there is high degree of diversity between and within breast cancer including angiogenic, invasive, and metastatic capacity, and all of these factors together determine the risk of cancer progression and therapeutic efficacy (4).

Although chemotherapy drugs are effective, these agents are cancer-nonspecific and can damage normal tissue. Side effects of such therapy can contribute to the high mortality of patients (5). Another problem of chemotherapy is the increased incidence of drug resistance. Therefore, the focus of cancer therapy today is the extensive effort to design the agents that selectively target cancer cells (6).

Host defense peptides (HDPs), as the natural compounds, are increasingly investigated in cancer treatment based on their cytotoxicity, but also the promising selectivity for cancer cells (7). In order to improve their therapeutic efficacy and decrease toxicity, many natural HDPs have been chemically modified (7).

Host defense peptides: frontline defenders in host

HDPs are small, evolutionarily conserved cationic molecules, with short amino acid sequences, produced by all living organisms, from microorganisms to mammals. In vertebrate, they are produced by the skin, mucosal epithelia and by innate immune cells (8, 9). HDPs usually have fewer than 50 amino acids and they are positively charged, with hydrophobic residues and amphiphilic properties thus interacting with negatively charged components of cell membrane (10) Natural peptides are classified into four structural groups due to their conformation: α-helical, β-pleated sheet, cyclic, and random coil HDPs (7).

HDPs are important components of innate immunity and their primary function is to protect the host from invading pathogens (9). The most important role of HDPs is their microbicidal effects (they were earlier named antimicrobial peptides (AMPs)). HDPs exhibit a broad spectrum of killing activity against bacteria, fungi and protozoa, without or with low cytotoxicity for normal mammalian cells, making them the suitable candidates for therapeutic use (11,12). Also, they can be involved in immunomodulation, endotoxin neutralization, chemotaxis and wound healing (12). Some HDPs show anti-diabetic effects, as they increase insulin secretion and improve glucose tolerance (13).

Anti-cancer activity of HDPs

Some of HDPs have anti-cancer activity regarding direct disruption of the cancer cell membrane, the induction of apoptotic cell death, as well as inhibition of angiogenesis and cancer cell proliferation (7). These peptides are of interest for use in cancer therapy, alone or in combination with other anti-cancer drugs, based on their capability to kill cancer cells as well as low toxicity against normal cells (14). To date, 3 257 antimicrobial peptides, including 251 peptides with anticancer activity, have been reported in the Antimicrobial Peptide Database (APD) (https://wangapd3.com/main.php).

The physical and chemical properties of HDPs greatly influence their tumoricidal efficacy. Numerous HDPs exhibit substantial selectivity for cancer cells (14). The negatively charged membrane of cancer cells, with phosphatidylserine, attracts positively charged HDPs. They accumulate on the plasma membrane, which leads to the formation of pores. Insertion of HDPs into the membrane leads to the membrane disruption or internalization of the peptide (15). HDPs can destroy the cell membrane by a few proposed mechanisms: a) The "barrel-stave" model (the accumulation of peptides on the cell surface, conformational changes and aggregation to form barrel-shaped multimers) (8). For example, this is the mechanism of action of peptide alamethicin, produced by the fungus *Trichoderma viride* (16); b) Carpet-model (HDPs cover outer layer of the cell membrane, and when the peptides concentration become critical, they cause phospholipids redirection). This model include the effects of cecropin P1, dermaseptin, and LL-37 peptides (8); c) Toroidal pore-model (in active form, at high concentrations, peptides are turn perpendicularly to the bilayer and destabilize the membranes with forming toroidal pore). It is a mechanism of action of peptides such as magainin-2, protegrin-1 and cecropin A (8); d) Other models include "sinking raft model", "molecular electroporation model", "aggregate channel model", etc. (8).

Programmed cell death (apoptosis) is initiated by two pathways: the extrinsic (receptor-mediated) and the intrinsic (mitochondria-mediated) (17). Both pathways are accompanied by the release of pro-apoptotic proteins from the mitochondria, such as the Bcl-2 family proteins and cytochrome c. Subsequent cascade activation of caspases, such as 8 and 9, leads to activation of caspase 3, which orchestrates the apoptotic cell death (17). Some HDPs have potential to diffuse into the mitochondria of cancer cell, causing the release of cytochrome c and apoptosis. (17). For example, dolastatin 10, derived from the Indian Ocean sea hare *Dolabella auricularia*, induces apoptosis in different cancer cell lines, including breast cancer (18). Also, pardaxin, initially isolated from marine fish (*Pardachirus marmoratus*), inhibits murine fibrosarcoma growth by inducing intrinsic apoptotic pathway in cancer cells (19). Cecropin B1 (CB1), initially derived

from cecropia moth *Hyalophora cecropia,* bound to luteinizing hormone-releasing hormone receptor ligand (LHRH'), causes ovarian and endometrial cancer cell death by membrane cell disruption and probably apoptosis (20).

Furthermore, some HDPs as immune defense regulators trigger the release of danger signals from dying cancer cells; enhance anti-cancer T cell immune responses, inhibit regulatory T cells functions and modulate chemokines expression (21). For instance, the hydrolysates of peptides from oyster *Crassostrea gigas* reduces growth of murine sarcoma, accompanied by strong immunostimulatory activity based on the enhanced natural killer (NK) cells activity, lymphocytes proliferation and macrophage phagocytosis (22). Also, β-defensin-2 efficiently inhibits cancer growth and increases chemotactic activity and maturation of dendritic cells in cancer microenvironment, thus triggering potent local cytotoxic T cells-mediated anti-cancer immune response (23).

In addition, certain HDPs have shown anti-angiogenic activity and thus also inhibit cancer progression. The human α-defensins, also known as the human neutrophil peptides (HNPs), can regulate angiogenesis by affecting the adhesion, proliferation, and migration of endothelial cells (24, 25). Xu et al reported that HNP-1 inhibits growth of human lung adenocarcinoma xenograft in nude mice by inducing cancer cell apoptosis, and reducing cancer angiogenesis (26).

HDPs of different origin as potential new trial weapons for breast cancer treatment

To date, plethora of bioactive peptides has been discovered from various organisms. Some of these effector peptides appear to be present as an alternative strategy to improve therapy of breast cancer.

Mammalian HDPs

Three subclasses of defensins have been identified as α, β, and θ, based on the disulfide linkages of cysteine residues (27,28). α-defensins are produced by neutrophils and Paneth cells in the ileum, while β-defensins are mainly produced by epithelial cells (27,28). The mechanism of defensins action includes disruption of the microbial cell membrane and metabolic disturbance (28). They also have considerable direct and selective anti-cancer effect (27). In addition, human epidermal growth factor receptor 2 (HER2), overexpressed in various cancers including breast cancer, coexists with human beta-defensine-1 (hBD-1) at the epithelial cell surface (29). Sun et al determined potential interaction between the hBD-1 peptide and HER2 (29). It appears that hBD-1 peptide interacts directly with HER2 receptor thus changing HER2 expression and signaling which gives the hope for its future use as an anti-cancer agent (29).

Bovine lactoferrin (bLf) is a natural protein with anti-cancer activity (30). Treatment with bLf inhibits growth of different breast cancer cell lines more efficiently than normal cells (31). It appears that bLf induces cell cycle arrest through downregulation of mammalian target of rapamycin (mTOR) signaling, thus indicating its potential to trigger nutrient/energy related cell stress (31). Similarly, human lactoferrin (hLf), decreases levels and activity of both cyclin-dependent kinase (Cdk)2 and Cdk4 and also blocks the transition from G1 to S phase of cell cycle (reviewed in 30). In addition, hLf induces apoptosis of breast cancer cells, through the decrease of Bcl-2 expression, release of cytochrome c and activation of caspases (reviewed in 30). Treatment with hLf increases NK cells-mediated cytotoxicity against breast cancer cells (reviewed in 30). Moreover, lactoferrin shows high selectivity for cancer cells and low toxicity for normal cells, even at high doses, and could be considered as a candidate for developing new therapeutic agents against breast cancer (30).

Lactoferricin B (LfcinB) is another peptide with broad spectrum of activities, obtained by pepsin digestion of bovine lactoferrin. Barragan-Cardenas et al showed that palindromic peptide LfcinB $(20\text{-}25)_{Pal}$ (^{1}RWQWRWQWR^{9}) and its analogues, have the significant cytotoxic effect on breast cancer cells MDA-MB-468 compared to the natural peptide (32). Treatment of MDA-MB-468 cells with the palindromic peptides induces morphological changes indicating apoptosis (32). Peptides have high affinity for breast cancer cells compared to normal fibroblasts thus indicating their selectivity (32). Dimeric peptide LfcinB $(20\text{-}30)_2$:(^{20}RRWQWR MKKLG^{30})$_2$-K-Ahx and its analogues exhibit marked cytotoxic effect on MCF-7 and HTB-132 triple-negative breast cancer cells. It appears that the dimeric peptide does not disrupt the integrity of the cytoplasmic membrane, but induces intrinsic apoptotic machinery in cancer cells (33). Moreover, Varagas-Casanova et al evaluated the cytotoxic effect of linear, dimeric, tetrameric and cyclic peptides derived from lactoferricin B, against MDA-MB-468 and MDA-MB-231 breast cancer cell lines (34). They have concluded that dimeric and tetrameric peptides LfcinB $(20\text{-}25)_2$ and LfcinB $(20\text{-}25)_4$, which contain ^{20}RRWQWRMKKLG^{30}, ^{17}FKARR WQWRMKKLGA^{31} and MKKLGA sequences have better cytotoxic effect then linear peptides (34). Free linear peptide LfcinB (20-25) shows cytotoxic effect and induces apoptosis, only delivered with liposome into the cytosol (34). The selected mammalian HDPs are summarized in Table 1.

Human cathelicidin antimicrobial peptide (CAMP) or human cationic antimicrobial peptide 18 (hCAP-18), encoded by the CAMP gene, have highly conserved N-terminal part containing cathelin domain (27). It can be found in neutrophils, monocytes, macrophages, NK cells, mast cells, dendritic cells and epithelial cells (27). Its active form named LL-37 is formed from a precursor peptide by the action of proteinases (27). The role of CAMP in cancer is controversial. It seems that LL-37 can exert either pro-tumorigenic or anti-cancer effects depending on specific cell surface receptors binding in different cell types and cancers. In fact, LL-37 has the ability to bind different cell receptors such as CXC chemokine receptor type 2 (CXCR2), N-formyl peptide receptor 2, insulin-like growth factor 1 receptor, purinoceptor 7 and HER2 (35). Overexpression of LL-37 in breast cancer cell, lung, ovarian and prostate cancer suggests that LL-37 might be involved in cancer progression (35). The

upregulation of HER2 with LL-37 in breast cancer cells leads to cancer cell proliferation (35). However, in colon cancer, gastric cancer and oral squamous cell carcinoma, LL-37 can exert anti-cancer effects (35). It has been shown anti-cancer effect of LL-37 regarding induction of caspase-3 mediated apoptosis via the P53-Bcl-2/BAX signaling pathway in oral squamous carcinoma cells (36). In addition, cathelicidin secreted from M1 macrophages induces Burkitt's lymphoma cell death by targeting mitochondria, in vitamin D-dependent fashion (37).

LL-37 stimulates migration of breast cancer cells trough activation of transient receptor potential cation channel subfamily V member 2 (TRPV2) and phosphatidylinositol-3-kinase/serine/threonine protein kinase B (PI3K/Akt) pathway (38). The decreased serum level of LL-37 after surgical removal of cancer indicated its potential as a biomarker for breast cancer (39). Chen et al have shown that CAMP receptor antagonist attenuates cancer growth mediated by tumor associated macrophages 2 (TAM2), indicating that CAMP could be a potential target to block the interaction between TAMs and cancer cells (39). Therefore, CAMP receptor antagonists may be a potential therapeutic option against breast cancer (39).

Amphibian HDPs

Amphibian skin is a very rich source of various HDPs with the broad spectrum of biological activities, including anti-microbial, anti-cancer, immunomodulatory and anti-diabetic effects (40). Till today, numerous natural amphibian peptides, in particular those derived from skin secretions of order Anura members (frogs and toads), and their synthetic analogues have been described as candidates for anti-cancer agents. Apart from the disruption of the integrity of plasma membrane and subsequent cancer cell death, some amphibian peptides can induce apoptosis via the mitochondrial pathway, but also inhibit tumor angiogenesis (40). Furthermore, there is a great potential of amphibian peptides to modulate host immune response in a complex manner (9,40). It appears that some of them exhibit immunostimulatory capacities which might be of relevance for the amplification of anti-cancer immune response (9,40). The selected amphibian HDPs with anti-cancer activity against breast cancer cells are summarized in Table 1.

Temporin-1CEa, derived from the Chinese brown frog *Rana chensinensis,* induces rapid cell death of MDA-MB-231 and MCF-7 human breast cancer cells (41). Temporin-1CEa induces phosphatidylserine exposure on the cell surface, increases plasma membrane permeability and leads to rapid depolarization (42). Cancer cell death is initiated with direct plasma membrane disruption and specific intracellular mechanisms, including Ca^{2+} leakage, reactive oxygen species (ROS) overproduction, and collapse of mitochondrial membrane potential (42). Due to its low cytotoxic effect on normal human umbilical vein smooth muscle cells and low hemolytic activity, temporin-1CEa appears to be a good candidate for anti-cancer therapy (41).

L-K6, an analog of naturally occurring temporin-1CEb initially isolated from the skin secretions of Chinese brown frog *Rana chensinensis, also* shows cytotoxic effect against MCF-7 cells, with relatively lower cytotoxicity against keratinocytes (43). L-K6 is imported in cell by clathrin-independent micropinocytosis (43). It causes destruction of the nucleus, without damaging the cytoskeleton and mitochondria (43). Wang et al showed that L-K6 inhibited P-glycoprotein (P-gP) function and reduced P-gP expression in doxorubicin resistant MCF-7/Adr cells (44). P-gP is a member of ABC transporters responsible for efflux of chemotherapeutic (44). L-K6, with downregulation of P-gP expression and inhibition of efflux with P-gP, improve the effect of doxorubicin in multidrug resistant (MDR) MCF-7/Adr cancer cells (44). L-K6 seems to be potentially powerful adjunct to classical MDR cancer therapy (44).

Brevinin-2R, isolated from marsh frog *Rana ridibunda,* is cytotoxic for few cancer cell lines, including MCF-7 breast cancer cells (45). Brevinin-2R can activate the lysosomal-mitochondrial death pathway through destabilization of lysosomal membrane and subsequent release of lysosomal cathepsins that can mediate caspase-independent cell death (45). It causes the decrease in mitochondrial membrane potential and increase ROS production, but not caspase activation (45). Cell death caused by brevinin-2R can be prevented by lysosomal membrane destabilization inhibitors and cathepsin inhibitors (45). However, MCF-7 cells that overexpress Bcl2, but not BNIP3 pro-apoptotic proteins, are resistant to the treatment with brevinin-2R (45).

Hymenochirin-1B was initially isolated from the Congo clawed frog *Hymenochirus boettgeri* (46). Increase in the cationicity of hymenochirin-1B while reducing helicity by substitutions with D-lysine, generated analogues with more potent cytotoxic activity against MDA-MB-231 than the native peptide, further accompanied by substantial hemolytic effects (46). Zhang et al have shown that hymenochirin-1B decreases mitochondrial membrane potential and increases the level of ROS and the expression of apoptosis associated proteins (Bax/Bcl-2 ratio and activated caspase-3) in lung cancer cell lines (47).

Hymenochirin-1Pa, HDP isolated from skin secretions of Merlin's clawed frog *Pseudhymenochirus merlini* (48), phylloseptin-TR peptides (1.1. TR, 2.2 TR and 3.1 TR), from Trinidadian leaf frog *Phyllomedusa trinitatis* (49), and analogues of alyteserin-2a from the midwife toad *Alytes obstetricans* (50), showed potent cytotoxic activity against MDA-MB-231 cells. Increase in the cationicity of the hymenochirin-1Pa by replacing amino acid residues, exhibited weak effects on antimicrobial and anti-cancer activity of the peptide, but it appears that D9k analog was not hemolytic (48). Phylloseptin-TR peptides show strong cytotoxicity for erythrocytes, which limits their potential use in cancer therapy (49).

As mentioned above, several amphibian HDPs exhibit considerable immunostimulatory effect which might be of

relevance for the enhancement of anti-cancer immune response. In this context, pseudhymenochirin-1Pb and pseudhymenochirin-2Pa are HDPs first isolated from skin secretions of Merlin's clawed frog *Pseudhymenochirus merlini* (51). Ps-1Pb and Ps-2Pb inhibit the production of immunosuppressive interleukin (IL)-10 and multifunctional IL-6 cytokines, while stimulating the production of pro-inflammatory IL-23 by murine peritoneal macrophages, which might be of relevance for the enhancement of anti-cancer immune response (51). Both peptides are cytotoxic against several cancer cell lines, including human breast cancer cells (51).

In addition, host-defense peptide frenatin 2.1 S, (and 2.2 S), isolated from skin secretions of Orinoco lime tree frog *Sphaenorhynchus lacteus,* can increase recruitment, activation and tumoricidal capacities of NK cells against 4T1 mouse breast cancer cells (52). Pantic et al showed that frenatin 2.1 S significantly increases the expression of inflammatory tumor necrosis factor-α (TNF-α) and IL-17 by murine peritoneal macrophages and T cells (52). Also, the analogues of alyteserin-2a inhibit the production of immunosuppressive transforming growth factor-β (TGF-β) and IL-10 by peripheral blood mononuclear cells, which might be exploited for the amplification of anti-cancer immune response (50).

HDPs from other animal species

The therapeutic potential, including anti-cancer activities, of the components of bee and wasp venoms (order *Hymenoptera*) has been investigated for decades. In this context, wasp venom derived peptide mastoparan showed membranolytic activity against few human carcinoma cell lines, including breast cancer cells, accompanied by low citotoxicity against human red blood cells and mammary epithelial cell (53). Hilchie et al have shown that mastoparan, capped with a C-terminal amide, directly kills human breast cancer cells, and acts synergistically with gemcitabine in mouse mammary carcinoma model (53). Further, HDP from bee venom named melittin, shows the anti-metastatic effects (54). Melittin suppress the EGF induced cell motility by blocking nuclear factor-κB (NF-κB) and PI3K/AKT/mTOR pathways and inhibiting focal adhesion kinase (FAK) phosphorylation and F-actin reorganization, in MDA-MB-231 and MFC-7 cells (54).

CM4 peptide has been isolated from the hemolymph of the Chinese silkworm *Bombyx mori.* It is an effective HDP against bacteria and fungi, acting by the disruption of the plasma membrane (55).Yang et al found that the peptide was highly cytotoxic for human breast cancer and leukemia cell lines, after fatty acid myristoyl conjugation (55). Their data suggested that fatty-acyl-conjugated CM4 have the ability to enter into cells and induce apoptosis. Breast cancer cells are more sensitive than normal cells to fatty-acyl-conjugated CM4 (55).

NRC-03 and NRC-07 peptides of pleurocidin family (found in the winter flounder *Pleuronectes americanus*) (56), were cytotoxic against breast cancer cells including drug-resistant and slow-growing breast cancer cells (57). The cytotoxic effect was examined using several human breast cancer cell lines including paclitaxel-resistant cells, as well as mouse breast carcinoma cells (57). These two peptides have the ability to disrupt the integrity of cell membrane and damage mitochondria thus inducing the release of cytochrome c (57). In addition, NRC-03 and NRC-07 enhance the efficacy of cisplatin against breast cancer cells (57). Intra-tumoral administration of NRC-03 and NRC-07 reduce murine breast cancer growth (57).

*Tachyplesin*s I, II and III, from the hemocytes of horseshoe crab *Tachypleus tridentatus*, and their cyclized analogues, efficiently bind negatively-charged cancer cell membranes with subsequent disruption of lipid bilayers (58). In breast adenocarcinoma cells, tachyplesin I was reported to induce late apoptosis/necrosis and also exhibits relatively low hemolytic activity (59).

Additionally, callyptide A, peptide from the Red Sea marine sponges, genus *Callyspongia*, inhibits the growth of MDA-MB-231 cells (60). Similarly, peptides derived from mucus of giant African snail *Achatina fulica* (61), as well as tissue hydrolysates of the Chinese three-striped box turtle *Cuora trifasciata* (62) exhibit substantial cytotoxic effect on MFC-7 cells. HDPs, mentioned above, are summarized in Table 2.

HDPs derived from plants

In addition to animals, plant HDPs, as essential components of innate immune system, has also been recognized as powerful weapon of host defense. Interestingly, defensin PvD1 from *Phaseolus vulgaris*, is highly cytotoxic towards human breast cancer cells when compared to HNP-1 (63). The peptide enters cancer cells and induces the apoptotic events (63). PvD1 is also a modulator of cell-to-cell adhesion (63). Targeting the cancer cell adhesion might be a possible strategy to prevent metastatic spreading.

Peptide MsDef1, from the seed of *Medicago sativa*, is an anti-fungal defensin which have the ability to bind glucosylceramide (GlcCer) (64). MsDef1 induces sphingomyelinase activity and enhances GlcCer levels in the resistant triple negative breast cancer cells (64). MsDef1 also releases ceramide which have the ability to modulate the biochemical and cellular processes that will lead to apoptosis (64). Addition of MsDef1 to the conventional chemotherapy with high dose of doxorubicin, exerts cumulative effects on cancer cell death (64). MsDef1 targets cancer specific thioredoxin (Trx) system and activates the apoptosis signal regulating kinase-1 (ASK1) pathway. Trx inhibitors are known to reactivate ASK1 pathway and sensitize cancer cells to chemotherapy, indicating that MsDef 1 might be suitable as potential adjuvant to anti-cancer therapy (64). Deoxybouvardin (RA-V) from plant *Rubia yunnanensis* exhibits cytotoxic effect on human and mouse breast cancer cells. This peptide acts through PI3K/Akt pathway (65). RA-V interrupts the interaction between phosphoinositide-dependent kinase-1

(PDK1) and Akt and induces apoptosis through inhibition of Akt activation in breast cancer cells (65). HDPs from plant species with anti-breast cancer effects, are listed in Table 2.

Table 1. Mammalian and amphibian HDPs with potential therapeutic use against breast cancer

Peptide	Source		Action against breast cancer cell	References
Human β-Defensin-1 (hBD-1)			Membrane cell disruption Interaction with HER2	28,29
Bovine Lactoferrin (bLf)			Cell cycle arrest Downregulation of mTOR signaling	30-34
Human Lactoferrin (hLf)			Cell cycle arrest in G1 to S transition Apoptosis Enhancement of NK cells activity	
Lactoferricin B			Apoptosis	
Temporin-1CEa	Chinese brown frog	*Rana chensinensis*	Enhancement of plasma membrane permeability Membrane depolarization ↑Ca2+,↑ROS	41,42
L-K6 (Temporin-1CEb analog)	Chinese brown frog	*Rana chensinensis*	Clathrin-independent micropinocythosis Destruction of nucleus without cytoskeleton and mitochondrial damage	43
Brevivin-2R	Marsh frog	*Rana ridibunda*	Destabilization of lysosomal membrane ↓Mitochondrial membrane potential ↑ROS production Autophagy-like cell death	45
Hymenochirin-1B	Congo clawed frog	*Hymenochirus boettgeri*	Cytotoxic activity Induction of apoptosis and cell cycle arrest via mitochondrial pathway	46,47
Hymenochirin-1Pa	Merlin's clawed frog	*Pseudhymenochirus merlini*	Cytotoxic activity	48
Phylloseptin-1.1TR, -2.1TR and -3.1TR	Trinidadian leaf frog	*Phyllomedusa trinitatis*	Cytotoxic activity	49
Alyteserin-2	Midwife toad	*Alytes obstetricans*	Cytotoxic activity ↓TGF-β, ↓IL-10	50
Pseudhymenochirin-1Pb (Ps-1Pb) and pseudhymenochirin-2Pa (Ps-2Pa)	Merlin's clawed frog	*Pseudhymenochirus merlini*	Cytotoxic activity ↓IL-10,↓ IL-6, ↑IL-23	51
Frenatin 2.1 S	Orinoco lime tree frog	*Sphaenorhynchus lacteus*	↑TNF-α, ↑IL-17 Enhancement of NK cells activity	52

Table 2. HDPs from different animal and plant species with potential therapeutic use against breast cancer

Peptide	Source		Action against breast cancer cell	References
Mastoparan (amidated)	Wasp venom	*Order Hymenoptera*	Membrane cell disruption	53
Mellitin	Bee venom	*Apis mellifera*	Anti-metastatic effects Inhibition of NF-κB and PI3K/Akt/mTOR signaling	54
Peptide CM4 (fatty-acyl-conjugated)	Chinese silkworm	*Bombyx mori*	Apoptosis	55
Pleurocidins: NRC-03; NRC-07	Winter flounder	*Pleuronectes americanus*	Membrane cell disruption Mitochondrial membrane damage	56,57
Tachyplesin I	Horseshoe crab	*Tachypleus tridentatus*	Late apoptosis/necrosis	59
Callyptide A	Red sea marine sponge	Genus *Callyspongia*	Cytotoxic activity	60
Peptides derived from mucus	Giant African snail	*Achatina fulica*	Cytotoxic activity	61
Peptides derived from tissue hydrolysates	Chinese three-striped box turtle	*Cuora trifasciata*	Cytotoxic activity	62
PvD1	Plant	*Phaseolus vulgaris*	Apoptosis Modulation of cell-to-cell adhesion and prevention of metastatic spreading	63
MsDef1	Plant	*Medicago sativa*	Apoptosis Cancer targeted sensitizer neoadjuvant to cancer therapy	64
Deoxybouvardin (RA-V)	Plant	*Rubia yunnanensis*	Apoptosis Inhibition of PI3K/Akt signaling	65

CONCLUSION

Future experimental and clinical research will tell if and when HDPs, as powerful "biological weapon", will become useful strategy for cancer therapy. It seems that natural HDPs and their synthetic analogues might be an inexhaustible source of valuable bioactive compounds. Due to considerable selectivity and specificity for cancer cells, low toxicity for normal cells, as well as immunomodulatory effects, there is a great interest of the therapeutic use of HDPs. Synthetic peptides or analogues of natural ones are increasingly created, with the aim of improving their natural abilities. It appears also that HDPs are potential candidates that may serve as vigorous and promising vaccine adjuvants that will enhance the antigen-specific immune response (66). The activation of interferon-γ (IFN-γ)-producing type 1 T helper cells and cytotoxic T cells is essential in cancer therapy and is a promising strategy for the development of modern adjuvants (67). Given the diversity and immunomodulatory capabilities of HDPs, they provide broad templates for designing effective innate defense regulator peptides, as cancer vaccine adjuvants (68).

Taken together, further identification of novel natural HDPs and their synthetic analogues, as well as the improvement of their anti-cancer capacities and selectivity, seems to be the valuable approach for the enhancement of therapy.

ACKNOWLEDGMENT

This work was funded by grant from the Faculty of Medical Sciences of the University of Kragujevac, Serbia (JP 10/18 and JP 16/19).

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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