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ANIMAL VENOM-DERIVED ANTIMICROBIAL PEPTIDES: NOVEL AND IMPROVED WEAPON FOR CANCER TREATMENT

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Abstract

Cancer is the second leading cause of human death worldwide. Conventional standard anticancer therapies such as chemo-, hormonal-therapies and radiation, are always accompanied with undesired severe side effects and toxicities due to their low specificity. Small peptides are being identified as potential anticancer agents as they could specifically target cancer cells without disrupting normal tissues, thus enable them to be a better alternative agent for the prevention and management of cancer. The increased expression of anionic phospholipids on cancer cell surface renders them more susceptible towards cationic antimicrobial peptides (AMPs). Knowing animal venoms could impair multiple hallmarks of cancer, AMPs isolated from animal venoms might be a new strategy for cancer treatment. In this review, we present the AMPs identified from animal venoms and discuss their multiple mechanism of action against cancer cells.

INTRODUCTION

Cancer is one of the leading causes of death and morbidity globally [1]. The incidence of cancer is increasing where the total of 55% of new cancer cases are from the developing nations in 2017 and this figure is expected to reach 60% by 2020 and 70% by 2050. Cancer also causes a substantial economic burden and human suffering; the cost associated with cancer cases worldwide was approximately US$1.16 trillion in 2010, the equivalent of >2% of the total global gross domestic product [2]. Estimated number of new cancer case in year 2018 was 18 million [3] with approximately 9.6 million deaths [1]. The most commonly diagnosed cancer types are breast, lung, colorectal, skin, prostate and stomach. Chemotherapy is one of the main treatment options. However, chemotherapy often results in the development of multi-drug resistance in cancer cells as well as damage of healthy cells [4]. Hence, adverse effects such as myelosuppression (decrease in blood cells production due to damaged bone marrow cells), alopecia (hair loss due to damaged hair follicles) as well as mucositis (gastrointestinal mucosal damage due to damaged intestinal mucus cells) are observed [5].

A growing body of peptides from animal venom has been demonstrated to possess physiological functions, such as antinociceptive [6], antiviral [7], antimicrobial [8], antibiofilm [8], immunomodulatory [9], anticancer [10], and analgesic activities [11]. Among these bioactive peptides, AMPs are extensively investigated as they hold the promise to hinder the ability of its targets to develop resistance and the possibility of targeting rapidly proliferating healthy cells [12, 13]. AMPs are short (5-50 amino acids) naturally occurring inducible effectors that participate in innate immunity of diverse organisms, with activity effective against fungi, bacteria and viruses [14]. They are characterized by an amphipathic features with a significant proportion of cationic and hydrophobic amino acid residues [15], as shown in **Figure 1**. Bacteria and some viruses present negative charge on their surfaces which contributes to the initial electrostatic interaction [16]. Similarly, cancer cells share similar characteristic of negative surface charge [16], which leads to the hypothesis that AMPs and anticancer peptides (ACPs) adopt a similar selectivity and mode of action theoretically, even though not all AMPs are ACPs, and *vice versa*. Bioactive peptides possessing cancer cells selectivity and penetration ability may be a resourceful strategy to overcome numerous challenges associated with existing therapeutics and bring revolution in anticancer drugs development. As studies regarding the bioactive peptides mechanism of action are primordial in optimizing drug development, this review will focus on the selectivity, efficacy, and mode of action of AMPs from animal venoms (**Table 1**) and discuss approaches described for enhancing their efficacy and selectivity towards targeted cells.

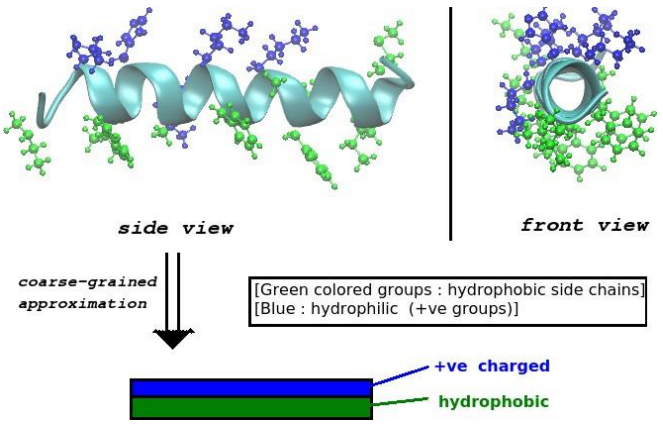

Figure 1. Three-dimensional structure of amphipathic AMP, Magainin II with hydrophilic (blue) and hydrophobic (green) halves, reproduced from reference [17]

ANIMAL VENOM

Animal venoms are intricate mixtures of enzymes, proteins and peptides which have been used as a traditional medicine among the Chinese for centuries. Venoms are classified based on origin (wasp venoms, bee venoms, snake venoms). Venoms have been constantly evolving through natural selection which makes them a valuable natural source for the development of new therapeutic agent.

Scorpion venom

Scorpions belong to the class of Arachnida of phylum Arthropoda, being one of the oldest arthropods. Scorpions inject the venom to their prey through telson, a bulb-shaped structure containing venom glands and stinger. Scorpions are divided into 18 families with more than 1,500 species [26]. This group of venom has been extensively studied for its anticancer potential.

Stigmurin

Stigmurin (Stig) is a peptide (17 amino acid residues) isolated from the venom gland of Brazilian yellow scorpion, *Tityus stigmurus* [27]. Studies showed that this peptide presented antimicrobial activity both *in vitro* and *in vivo* [28, 18]. At the same time, Stig showed very weak antiproliferative effect against human cervical carcinoma (SiHa) and green monkey kidney (Vero E6) cells, and moderate effect against human hepatocellular carcinoma (HepG2) and canine kidney epithelial (MDCK), with low hemolytic activity [18, 29]. However, more extension to various tumorigenic and non-tumorigenic cell lines should be conducted in time to come to suggest the anticancer potential and selectivity of Stig. Parente et al. [19] modified peptide Stig by altering the amino acid sequence, presenting two peptide analogs StigA6 and StigA16 with higher net charge and hydrophobic moment via lysine (Lys) substitutions.

Both analogs were reported to exhibit greater antiproliferative activity against human renal cell adenocarcinoma (786-0), mouse melanoma (B16F10), human cervix adenocarcinoma (HeLa) and human pancreas adenocarcinoma (panc 10.05) with lower toxicity against normal mouse fibroblast cell line (NIH/3T3) compared to Stig. The resultant change in the antiproliferative activity suggests that Lys might direct the binding of bioactive peptides to anionic cancer cell membranes, instead of normal cells. Support for this comes from a finding on the strong preferential binding of Lys to anionic membranes by Yang et al. [30]. However, the mechanism of action of these peptides was not outlined. These findings suggest that amino acid modification increasing the overall net charge and hydrophobicity could possibly enhance their interaction with cancer cell membranes, thus resulting in cell death.

Table 1. List of AMPs derived from animal venoms and their mode of action

Peptide Name	Source	*In vitro* cancer cells and *in vivo* tumor models*	Mode of action	Ref.
Scorpion venom				
Stigmurin (Stig)	*Tityus stigmurus* (Brazilian yellow scorpion)	SiHa	Unclear mode of action	[18]
StigA6, Stig A16		786-0, B16F10, HeLa, panc 10.05	Unclear mode of action	[19]
BmKn-2	*Mesobuthus martensii Karsch*	HSC-4, KB, SW620	Apoptosis via p53 activation, promoting Bax expression and activation of caspase -3, -7 and -9	[35, 33]
Spider venom				
Lycosin-I	*Lycosa singoriensis*	HCT-116, HT1080, DU145, HepG2, A549*, H1299*, HeLa*, PCa	Apoptosis via caspase-3 activation; Inhibition of cell proliferation via p27 upregulation; Apoptosis and inhibition of cell migration via STAT3 pathway inactivation	[42, 43]
R-lycosin-I		A549, PC-3, HeLa, MDA-MB-231	Apoptosis via caspase-3 activation; Inhibition of cell proliferation via p27 upregulation	[50]
Lycosin-II		HCT-116	Apoptosis via upregulation of Bax expression and activation of caspase -3; Disruption of membrane via pore formation	[10]
Bee and wasp venom				
Crotamine	*Crotalus*	B16-F10*,	DNA-binding;	[59,

	durissus terrificus	SK-MEL-28, MIA PaCa-2	Calcium mobilization; Mitochondrial depolarization	60]
Mastoparan	*Vespa cabro*	B16F10-Nex2*	Apoptosis via caspase-3, -9, -12 activation, PARP cleavage, Bax and Bim upregulation, Bcl-XL downregulation	[20]
Mastoparan -C (MP-C), cMP-C, tMP-C		H157, MDA-MB-435S, MCF-7, U251-MG, PC-3	Unclear mode of action	[73]
Melittin	*Apis mellifera*	SKOV-3, PA-1, A549, CaSki, MCF-7, MDA-MB-231, B16F10, A375SM, SK-MEL-28	Apoptosis via death receptor-3, -4 and -6 upregulation as well as STAT3 pathway inactivation; Inhibition of tumor angiogenesis and progression via HIF-1 and VEGF suppression; Inhibition of cancer cells invasion and migration via P13K/Akt/mTOR pathway suppression	[21, 22, 23, 24, 25]
Decoralin-NH2	*Oreumenes decorates*	MCF-7	Membrane disruption	[95]

Abbreviations: SiHa, human cervical cancer cell line; 786-0, human renal cancer cell line; B16F10, murine melanoma cell line; HeLa, human cervical cancer cell line; panc 10.05, human pancreatic cancer cell line; HSC-4, human oral squamous cancer cell line; KB, human mouth epidermoid cancer cell line; SW620, human colon cancer cell line; HCT-116, human colon cancer cell line; HT1080, human fibrosarcoma cell line; DU145, human prostate cancer cell line; HepG2, human liver cancer cell line; A549, human lung cancer cell line; H1299, human lung cancer cell line; PCa, human prostate cancer cell line; MDA-MB-231, human breast cancer cell line; SK-MEL-28, human melanoma cell line; MIA PaCa-2, human pancreatic cancer cell line; B16F10-Nex2, murine melanoma cell line; H157, human lung cancer cell line; MDA-MB-435S, human breast cancer cell line; MCF-7, human breast cancer cell line; U251-MG, human glioblastoma cell line; PC-3, human prostate cancer cell line; SKOV-3, human ovarian cancer cell line; PA-1, human ovarian cancer cell line; CaSki, human cervical cancer cell line; A375SM, human melanoma cell line.

BmKn-2

BmKn-2 is an alpha-helical cationic peptide derived from the venom of scorpion *Mesobuthus martensii* Karsch with potent and broad spectrum antimicrobial activity, including multidrug-resistant strains of *Neisseria gonorrhoeae* [31, 32]. Investigation by various studies reported the selective potent cytotoxicity of BmKn-2 on human oral squamous carcinoma (HSC-4), human colon carcinoma (SW620), and human mouth epidermoid carcinoma cells (KB) [33, 34, 35]. Selectivity of BmKn-2 was confirmed as low toxicity was detected on dental pulp stem cells, red blood cells, dental pulp cells (DPC), and normal gingival cells (HGC) [34, 33]. Mechanism of BmKn-2 was further investigated whereby it induced intrinsic apoptotic pathway by inducing the expression of p53, followed by inducing Bax expression and suppressing Bcl-2 expression in cancerous cells [35, 33]. Moreover, upregulation of initiator caspase-9 and executor caspases-3 and -7 in HSC-4 cells have been observed. In contrast, BmKn-2 upregulates Bcl-2 expression but downregulates Bax and Caspase-3 expressions in HGC and DPC [33]. It was suggested that the toxicity and selectivity of BmKn-2 are likely due to the net negative charge of cancer cell membrane. On the contrary, normal eukaryotic cell surface exhibited an overall neutral charge owing to the presence of zwitterionic phosphatidylcholine (PC) predominantly on the outer leaflet while negatively charged phosphatidylserine (PS) preferentially localized on the cytoplasmic leaflet of plasma membrane, thus reducing electrostatic attraction between cationic peptides and membrane [36]. Apart from the cationic nature, the amphipathic secondary structure of BmKn-2 containing hydrophilic and hydrophobic regions was believed to be critical in the membrane insertion and disruption of cancer cells [34], which is in agreement with the mechanism of action of ACP melittin [37]. Thus, these evidences indicate that BmKn-2 warrants further in vivo investigation as potential candidate for clinical trials.

Spider venom

Spider venom consists of a complex mixture of organic components, proteins, and neurotoxins [38]. More than 1000 different peptides can be found in the venom of certain species [39]. Approximately over 10 million bioactive peptides can be possibly presented in spider venoms [40].

Lycosin-I

Lycosin-I is an AMP with alpha-helical conformation isolated from the venom of *Lycosa singoriensis* [41]. Liu et al. [42] demonstrated that this peptide induced apoptosis in HeLa, colon adenocarcinoma (HCT-116), fibrosarcoma (HT1080), prostate carcinoma (DU145), lung adenocarcinoma (H1299, A549), and HepG2 via caspase-3 activation as well as upregulation of p27 protein to inhibit cell proliferation. In terms of *in vivo* studies, mice xenograft models bearing HeLa, H1299 and A549 cells showed suppressed tumor growth. Shen et al. [43] indicated that lycosin-I induced apoptosis and inhibited migration of prostate (PCa) cancer cells at high and low concentration, respectively through inactivation of STAT3 signaling pathway. Nevertheless, low potency and cellular entry of lycosin-I in tumor cells limited its applicability as anticancer therapeutic agent. In a recent study, Tan et al. [44] reported the mechanism of membrane/lycosin-I interaction by using total internal reflection fluorescence microscopy. Lycosin-I gradually aggregated onto the lipid membranes which subsequently induced the formation of amphipathic helix formation of lycosin-I that may facilitate their penetration into the cell [44].

To further enhance the cellular uptake and tumor penetration, lycosin-I-conjugated gold nanoparticles was constructed. By conjugating lycosin-I to the surface of gold nanoparticles, the intracellular internalization was increased significantly, possibly via clathrin- and caveolae-mediated endocytosis, along with excellent selectivity over noncancerous cells [45]. Alternatively, the cellular uptake of lycosin-I can be optimized by site-targeted modification. Mutant R-lycosin-I was designed by substituting Lys with arginine (Arg) to increase

overall hydrophobicity, thus exhibited greater anticancer effect as well as tumor penetration [46]. Extensive studies have been carried out and reported the guanidinium group of Arg was closely associated with its penetrating ability due to the affinity of phospholipid polar head groups [47, 48, 49].

Furthermore, R-lycosin-I adopted the same mode of action as lycosin-I in inducing cell death in A549, HeLa, human breast adenocarcinoma (MDA-MB-231), and human prostatic carcinoma (PC-3) cell lines. Thus, amino acids substitution can increase peptides specificity and efficacy against cancer cells without altering the mechanism of action. Considering that amino acid modification greatly improved the anticancer effect and tumor penetrating ability of lycosin-I, Zhang et al. [50] further optimized R-lycosin-I by monosaccharides conjugation. As a result, monosaccharides facilitated the binding of R-lycosin-I-monosaccharide conjugate to gluocose transporter 1 (GLUT1), due to the fact that GLUT1 is highly expressed in various cancer cells, thus enhancing its enrichment on the cell surface to induce cell death [50]. Furthermore, depolarized mitochondrial membrane potential and release of lactate dehydrogenase (LDH) were observed, indicating R- lycosin-I-monosaccharide conjugate exerted its cytotoxicity by modulating mitochondria- dependent apoptotic pathway and membrane disruption.

While *in vitro* study showed great promise, intraperitoneally- and intratumorally-injected A549-luciferase tumor xenograft with R-lycosin-I-monosaccharide conjugate produced parallel results as that of *ex vivo* 3D tumor spheroids of A549 cells, where the tumor growth was remarkably suppressed. However, the poor perfusion of tumor inner regions and relatively rapid clearance of peptides from the tumor site resulted in the incomplete removal and constant growing of these tumors following intratumor and intraperitoneal injection, respectively [51, 52]. Regardless of the fact that the tumors may disappear without any further treatment in weeks to months in some cases [53, 54, 55], further modification of R-lycosin-I-monosaccharide conjugate is needed to impart clinically therapeutic index. Overall, the established conjugate display excellent potential in cancer-targeted therapy.

Lycosin-II

Recently, a novel AMP, lycosin-II isolated from the same spider venom with amino acids sequence differs from that of lycosin-I was reported to exhibit cytotoxicity and antiproliferative activity against HCT-116 cancer cells through dual mechanisms of apoptosis induction and membrane disruption [10]. They have further demonstrated that lycosin-II increased the expression level of Bax and resulted in caspase-3 activation rapidly within an hour. Moreover, a cytosolic enzyme, LDH was released from HCT-116 and pores were formed on cell surface after incubation for only 30 minutes with lycosin-II. Whether lycosin- II induced apoptosis or membrane lysis to kill cancer cells as the main mechanism of action and the rationale of their coexistence still remain unknown, however, could enable greater anticancer efficacy. Unfortunately, the moderate hemolytic activity of lycosin-II against human red blood cells limited its potential as potential anticancer agent [10].

Snake venom

Snakes are known to produce deadliest venom. However, some of them are harmless. Researchers have reported that the venom toxicity differs among species, age, habitat or even climate [56]. Snake venoms are rich source of bioactive peptides for potential anticancer treatment with some currently being evaluated in clinical studies [57].

Crotamine

Crotamine was the first venom-derived peptide with natural cell-penetrating, broad spectrum antimicrobial and remarkable antifungal properties [58]. The toxicity of crotamine against murine melanoma (B16-F10) cells, human melanoma (SK-MEL-28) cells and human pancreatic carcinoma (MIA PaCa-2) was examined [59, 60]. Unlike other ACPs, crotamine mainly targets primary lysosome and mitochondria, thereby causing rapid release of intracellular calcium and mitochondrial depolarization [60]. Furthermore, the selectivity of crotamine for tumor cells is promising as demonstrated by effective localization of crotamine in B16-F10 cells and mice engrafted with B16-F10 subcutaneous melanoma [59, 60]. Fluorescence imaging revealed significant fluorescently labelled cromatine uptake and accumulation in tumor cells harbouring necrotic areas and rapidly proliferating metastatic cells without apparent detectable signal of crotamine in surrounding normal cells. Furthermore, the authors tracked the retention time of crotamine in these cells using fluorescent dye and demonstrated long retention as reflected by fluorescence retained by at least 70% cells after 24 hrs [60]. These results suggested that crotamine could be a potential tumor-specific imaging agent, metastasis marker and cytotoxic agent.

Crotamine apparently does not require assistance for their uptake into the tumors because it possesses efficient penetration ability by ubiquitously crossing cell membranes via receptor- and energy- independent mechanisms. In fact, crotamine interacts with extracellular matrix proteoglycans followed by clathrin-mediated endocytosis [61, 62]. After 21 days of treatment with crotamine, successful delay of tumor implantation, inhibition of tumor growth and increase of mice lifespan were observed in B16-F10-bearing mice [59]. Additionally, histopathological evaluation of long-term crotamine administration evidenced no toxicity in kidney and liver tissue sections, as well as immunotoxicity in melanoma-bearing mice [60]. However, study regarding the local and systemic safety of crotamine in biological models demonstrated by Silvestrini et al. [63] recently suggested its adverse effects. Data showed that crotamine elicited an inflammatory response, as confirmed by the production of pro-inflammatory cytokine (TNF-α), C-reactive protein (CRP) and nitric oxide in Wistar rats (*Rattus norvegicus albinus*) treated with crotamine.

Furthermore, anti-inflammatory (IL-10) cytokine was also produced to trigger anti-inflammatory functions, thereby balancing inflammation to mount effective T-cells responses [64]. However, the anti-inflammatory activity was intermittent as upregulation of N-acetylglicosaminidase, a macrophage activity biomarker was observed [63]. The neglected anti-inflammatory response and initiation of an inflammatory environment have resulted in further investigation of oxidative stress commonly associated with inflammatory responses. Results suggested that crotamine induced redox-imbalance as reflected by increasing serum levels of thiobarbituric acid reactive substances (TBARS) and decreasing sulfhydryl groups. Considering the inflammatory effect associated with crotamine, the clinical use of protamine in its original form is limited [63].

Bee and wasps venom

Among the arthropods, bee venom (BV) is extensively studied due its anticancer activity. BV and wasp venom (WV) contain complex mixtures of components such as peptides, amines and enzymes [65, 66, 67]. Many scientific studies regarding the toxicity and mode of action of BV, WV and their peptides towards cancer cells have been published due to its promising results [68].

Mastoparan

Mastoparan, a 14-residues peptide isolated from *Vespa cabro* wasp venom [69], showed antimicrobial activity against colistin-resistant *Acinetobacter baumannii* [70] and exocytotic activity in various cell types by inducing phospholipase C and A2 activation [71, 72]. The anticancer activity of mastoparan and its analogues was also examined, where their selective efficacy in *in vitro* and *in vivo* models were confirmed [73, 74, 20]. Cytotoxicity of mastoparan against human leukemia (Jurkat, and THP-1), murine myeloma (HOPC), mouse mammary carcinoma (4T1), and human breast carcinoma (MDA-MB-231, MDA-MB-68, SKBR3, and T47D), including slow-growing and paclitaxel-resistant MCF7-TX400 breast cancer cells was demonstrated [74]. In order to promote peptide stability, chemical modification of Mastoparan has been employed by amidating its C-terminal (Mastoparan-NH_2), where its potency against Jurkat cells was remarkably increased due to greater conformational propensity to adopt active conformation [74], which is in contrast to the non- amidated mastoparan [20]. This is in agreement with a study by da Silva et al. [75] reporting C-terminal amidation of mastoparan analog promoted stabilization of α-helix conformation, thereby permitting deeper interaction with the cell membranes. In addition to improved anticancer potency, Mastoparan-NH2 elicited different killing mechanism involving membranolytic activity [74] which explains its direct mechanism acting independent of cells proliferative capacity rather than apoptosis as reported for its non-amidated analog [20].

In a recent study by Chen et al. [73] demonstrated increased cytotoxicity of mastoparan analog, mastoparan-C with chemical modifications (head-to-tail cyclization, and N-terminal extension with a short cell-penetrating peptide, TAT sequence) against non-small cell lung cancer (H157), PC-3, human breast adenocarcinoma (MCF-7), human glioblastoma astrocytoma (U251-MG), and human breast ductal carcinoma cell lines (MDA-MB-435S), where the cyclized analog was markedly more potent and specific than the parental peptide. Previous studies have reported that the stability of peptides could be enhanced with cyclization of N- and C-terminal cysteine residues via disulfide-bridge to restrict conformational flexibility of linear peptides and to prevent proteases degradation [76, 77]. However, contradicting results was reported where cyclized mastoparan-C showing weaker serum stability than parental mastoparan-C [73]. Whether cyclization and N-terminal extension of mastoparan-C induced cell death through apoptosis or membranolysis has not been evaluated. Besides, mastoparan alone was able to notably delay tumor growth, suppress tumor progression and prolong the survival of mice bearing murine melanoma B16F10-Nex2 tumor [20]. Apart from that, synergistic effects of mastoparan when used with anticancer drugs etoposide and gemcitabine were demonstrated in Jurkat cells and highly aggressive 4T1- bearing mice, respectively [74].

In comparison to mastoparan-treated mice, the tumor mass and volume were significantly reduced when administered with both mastoparan and gemcitabine [74], thereby suggesting that mastoparan may be a potential chemo-sensitizing agent against breast cancer. The synergism shown could minimize the side effects of anticancer drugs on normal cells and enhance their anticancer effect as lower drug concentration is needed to achieve remarkable anticancer effects both *in vitro* and *in vivo*. Nonetheless, the immune response and mechanism responsible for the synergy between mastoparan and gemcitabine *in vivo* need to be further elucidated [74].

Melittin

Melittin, a cationic amphipathic peptide with 26 amino acid residues is a major component of European honey bee (*Apis mellifera*). Similar to mastoparan, substantial studies have been carried out to demonstrate the biological activities of melittin such as antimicrobial [78], anticancer [79, 80] and antiviral [81]. The cytotoxicity and mechanisms of melittin on malignant cells have been extensively studied whereby growth and proliferation inhibition were reported [82, 23, 21, 22]. Further studies showed that the aforementioned peptide induced death-receptor-mediated apoptosis in ovarian cancer cells (SKOV-3 and PA-1) via upregulation of death receptor-3, -4 and -6 and inhibition of STAT3 pathway [24]. Alternatively, melittin induced the activation of mitochondrial-associated apoptotic signalling as demonstrated in human gastric cancer cells (SGC-7901) [83]. In addition, melittin regulated angiogenesis and tumor progression by suppressing epidermal growth factor (EGF)- induced hypoxia inducible factor-1 (HIF-1) and vascular endothelial growth factor (VEGF) protein expression in cervical carcinoma (CaSki), A549 cells and A549 tumor-bearing mice [22, 23]. Furthermore, Jeong et al. [21] suggested that melittin inhibited motility and invasion of MCF-7 and MDA-MB-231 cells by suppressing EGF-induced MMP-9 and FAK mediated by PI3K/Akt/mTOR signalling pathway.

Similarly, recent study reported that melittin inhibited the progression of B16F10 and human melanoma (A375SM and SK-MEL-28) cells by suppressing PI3K/Akt/mTOR and MAPK signalling pathways [25]. Activation of both pathways are closely associated with the development of malignant melanoma by regulating tumor cellular processes such as cell proliferation and metastasis [84, 85]. Moreover, melittin sensitized the anticancer effect of temozolamide (chemotherapy agent) in highly chemoresistant metastatic melanoma cells [25], which is in consistent with earlier studies indicating sensitization of melanoma cells to temozolamide via inhibition of PI3K/Akt/mTOR signalling [86, 87]. However, application of melittin *in vivo* is restricted as it possesses wide-spectrum lytic activity including erythrocytes [67, 88, 89]. Hence, recombinant immunotoxin or nanoparticles were developed to overcome this adverse effect [90, 91, 92]. Su et al. [93] designed a fusion protein containing the amino-terminal fragment (ATF) of Urokinase plasminogen activator (uPA) and melittin to compete against uPA for uPAR binding to reduce non-specific toxicity of melittin on normal cells. This fusion protein was found to selectively induce G1 phase cell cycle arrest, growth inhibition as well as apoptosis in SKOV-3 cells selectively.

Decoralin

Decoralin (Dec) is a novel cationic α-helical peptide derived from *Oreumenes decoratus* wasp venom. Its amidated analog, Dec-NH_2 was reported to exhibit greater antimicrobial activity than parental peptide Dec against yeast, Gram-positive and Gram-

negative bacteria [94]. Nevertheless, both Dec and Dec-NH2 displayed pronounced lytic activity against erythrocytes which limited its therapeutic application. Recently, a Dec-NH2 peptide have been chemically modified via leucine substitutions and demonstrated selective cytotoxicity against MCF-7 cells with greater resistance to degradation and lower haemolytic effect than its parental peptide [95]. Similar effect was observed in Arg substituted Dec-NH2, possibly due to improved membrane permeation [96]. It is evidenced by the cell penetrating abilities of Arg- rich peptides which could be partly contributed by the affinity of guanidinium group to the phospholipid polar head groups [97, 98, 47].

CONCLUSIONS

The use of multifunctional AMPs of as anticancer agent has become more prevalent in recent years. Multiple examples discussed in this review emphasize the importance of rational design of animal venom-derived bioactive peptides for the development of highly efficacious and selective anticancer agent. Their activity, stability and selectivity can be further optimized by incorporating various modifications such as bioconjugation, cyclization, amidation, and amino acid alteration. Furthermore, combination therapy of existing anticancer drugs with venom-peptides has demonstrated optimal anticancer effectiveness. Taking advantage of the difference between normal and tumor-specific membrane proteins allow the improvement of bioactive peptides specificity. Understanding their interactions and mechanism of action will further help facilitating the complete utilization of venom-based peptides clinically.

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