Pleiotropic Anticancer Properties of Scorpion Venom Peptides: *Rhopalurus princeps* Venom as an Anticancer Agent

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Abstract: To date, the success of conventional chemotherapy, radiotherapy, and targeted biological therapies in cancer treatment is not satisfactory. The main reasons for such outcomes rely on low target selectivity, primarily in chemo- and radiotherapy, ineffectiveness to metastatic disease, drug resistance, and severe side effects. Although immune checkpoint inhibitors may offer better clinical promise, success is still limited. Since cancer is a complex systemic disease, the need for new therapeutic modalities that can target or block several steps of cancer cell characteristics, modulate or repolarize immune cells, and are less toxic to healthy tissues is essential. Of these promising therapeutic modalities are pleiotropic natural products in which scorpion venom (SV) is an excellent example. SV consists of complex bioactive peptides that are disulfide-rich of different peptides’ length, potent, stable, and exerts various multi-pharmacological actions. SV peptides also contain ion channel inhibitors. These ion channels are dysregulated and overexpressed in cancer cells, and play essential roles in cancer development and invasion, as well as depolarizing immune cells. Furthermore, SV has been found to induce cancer cell apoptosis, and inhibit cancer cells proliferation, invasion, metastasis, and angiogenesis. In the current review, we are presenting data that show the pleiotropic effect of SV against different types of human cancer as well as revealing one potential anticancer agent, *Rhopalurus princeps* venom. Furthermore, we are addressing what is needed to be done to translate these potential cancer therapeutics to the clinic.

Keywords: angiogenesis, apoptosis, cell arrest, cytotoxicity, immunomodulation, ion channels, metastasis, polarization

Introduction

Cancer is a devastating term that people afraid to hear; besides, it is the second leading cause of death worldwide. The incidence of cancer cases worldwide is on the rise, where 17 million new cases of cancer were diagnosed in 2018, and 9.6 million people died because of cancer in the same year. Furthermore, cancer incidence is projected to increase by 62% in 2040. Thus, the future burden will increase yearly as the numbers, and the projections are apparent.

Cancer development is a multistep process and it starts with an initiation step involving DNA mutation. Such mutation could either be inherited or acquired following chronic inflammation, exposure to chemicals or viruses yielding to molecular events that lead to cellular transformation. This transformation gains the abnormal cell(s) specific characteristics. Of these characteristics: 1) evading immunosurveillance and
programmed cell death; 2) becoming dysregulated regarding specific genes, proteins and ion channel expression, and go through uncontrolled cell proliferation; 3) stimulating and sustaining new blood vessels, angiogenesis, for their growth; and 4) ability to invade tissues and metastasize to other locations in the body. In addition, the immune cells that are recruited to tumor site become depolarized and lose their normal function and may become enhancers for tumor growth. All in all, these changes yield to the development of a heterogeneous tumor microenvironment.

To date, the success of conventional chemotherapy, radiotherapy, and targeted biological therapies in cancer treatment is not satisfactory. The main reasons for such outcomes rely on low target selectivity, primarily in chemo- and radiotherapy, ineffectiveness to metastatic disease, drug resistance, and severe side effects. In contrast, immune checkpoint inhibitors are now showing better promising results. However, success in cancer therapy is still limited. Since cancer is a complex systemic disease, the need for new therapeutic modalities that can target or block several steps of cancer cell characteristics, modulate or repolarize immune cells, and are less toxic to healthy tissues is essential. Of these promising therapeutic modalities are pleiotropic natural products in which they are multi-targeting and have fewer side effects. An excellent example of a pleiotropic natural product is the scorpion venom (SV). SV consists of complex bioactive peptides that are disulfide-rich of different peptides’ length, potent, stable, and exerts various multi-pharmacological actions (Figure 1). Research studies have demonstrated that SV peptides block/modulate ion channels. These ion channels are dysregulated and over-expressed in cancer cells and play essential roles in cancer development and invasion, as well as depolarizing immune cells. Furthermore, SV has pleiotropic anticancer activities such as inducing apoptosis, and inhibiting cancer cells proliferation, invasion, metastasis, along with angiogenesis. Besides, substantial evidence exists that SV is an immunomodulator. In the current review, we are presenting data that show the pleiotropic effect of SV against different types of human cancer as well as revealing one potential anticancer agent, *Rhopalurus princeps* venom. Furthermore, we are addressing what is needed to be done to translate these potential cancer therapeutics to the clinic.

**Ion Channels and Cancer Cells**

It has been proven that tumor cells use ion channels to support their proliferation, adhesion and invasion processes. These processes occur by ion channels interplay with changes in cell morphology and volume. In particular, this has been shown to be the mechanism of glioma cell growth and invasion mainly via the electrochemical efflux of Cl\(^-\) and K\(^+\) as KCl and water, which is mediated by an increase in intracellular Ca\(^{2+}\) ions, leading to cell shrinkage that enables the cell to invade the brain tissue.

Furthermore, expression of ion channels has been demonstrated to be altered, dysregulated, or overexpressed in several types of cancers. For instance, chloride channels have been found to be dysregulated in multiple cancer types; either downregulated such as human colorectal cancer or altered as in glioma to regulate glioblastoma.

![Figure 1](https://www.dovepress.com/submit-your-manuscript) The multi-pharmacological actions of scorpion venom bioactive peptides on cancer cells.
On the other hand, potassium channels such as voltage-gated potassium channels (Kv) and calcium-activated potassium channels (KCa) and their subtypes have been overexpressed or dysregulated in multiple cancers including glioblastoma, breast, colon, prostate cancer and lymphoma. Similarly, voltage-gated sodium channels (VGSCs; Nav1.5, Nav1.6, Nav1.7) have been found to be overexpressed in astrocytoma, breast, colon, cervical, prostate and non-small cell lung cancers. Lastly, calcium channels as voltage-gated Ca$^{2+}$ channels or transient receptor potential (TRPC) ion channels are overexpressed in breast, liver, stomach, ovarian and glioma tumors. Furthermore, when these Ca$^{2+}$ channels were silenced, tumor proliferation is reduced.

SV is a complex mixture of substances, among which are inorganic salts, free amino acids, heterocyclic components, peptides, and proteins. The most distinctive component of SV is peptides that modify, modulate or block ion channels, specifically modify the gating mechanism of Na$^+$-channels, block K$^+$-channel and Cl$^-$ or modulate the function of calcium channel. Thus, this opens the implication of using SV peptides in cancer therapy and development research to evaluate their potential in the clinical setting.

**Anticancer Effects of SV and Their Peptides**

**Proliferation Inhibition and Inducing Apoptosis**

Several species-specific SV have been tested in preventing or blocking the proliferation of several types of human cancer cell lines (Table 1). This anti-proliferative mechanism of action of SV or their purified peptides can be summarized into inducing apoptosis, cell arrest at Go/G1 phase, and inhibition of DNA synthesis.

Several studies have shown that the whole SV from *Androctonus crassicauda*, *Androctonus bicolor*, *Leirus quinquestriatus*, *Odontobuthus doriae*, and *Rhopalurus junceus* species reduced proliferation of human brain, breast, colorectal, lungs, cervix, and larynx cancer cell lines (Table 1). However, *Rhopalurus junceus* whole SV was tested against hematopoietic tumor and lymphoma cell lines and showed no activity. Regardless of the latter, the reduction of proliferation in the above cancer cell lines was due primarily to inducing apoptosis specifically by increasing the expression of apoptotic gene *bax*, and *bax/bcl* (anti-apoptotic gene) ratio, reducing *bcl* gene expression, increasing caspase enzymes 3, 8, 9 activities, and inducing Fas-L expression.

Furthermore, SV from either *Androctonus bicolor* or *Androctonus crassicauda* has shown to arrest cancer cells in S phase, Go/G1 phase or downregulating cyclin genes expression. Following the improvement of proteomic approaches to study peptides and their anticancer activities, a high throughput platform has been utilized to identify a panel of novel potential anticancer peptides in scorpion venoms and to predict their putative functions and targeted signaling pathways. Li et al have demonstrated a panel of potential anticancer peptides from *Androctonus mauritanicus* and *Androctonus australis* venoms and from these, Goneastestide, a peptide from *Androctonus mauritanicus*, has been found to exhibit the best anti-proliferative activity on human colon cancer cell line HCT116 by modulating cell cycle checkpoint proteins (Table 1). Similarly, in vivo administration of SV from *Androctonus amoreuxi* reduced Ehrlich ascites carcinoma and solid Ehrlich tumor size, and increased survival of mice-bearing intraperitoneal Ehrlich carcinoma as well as topical administration of *Leirus quinquestriatus* SV reduced skin carcinogenesis incidence in mice.

Iberiotoxin (IbTX) is a toxin isolated from *Buthus tumulus* scorpion and has been found to inhibit calcium-activated potassium channels (KCNMA1). These potassium ion channels were found to be over-expressed in different cancer types such as glioma, as well as hormone-influenced like breast, prostate, ovarian and cervix cancer. It has been shown that iberiotoxin decreases HeLa cell proliferation and human ovarian cancer (A2780) cell lines (Table 1).

Several types of peptides from *Buthus martensi* Karsch (BmK) have been tested in vitro and in vivo models. In one study, a peptide initially identified as 70–80 kDa fraction, termed by fractionation SVCIII, has shown anti-proliferative effect on human acute monocytic leukemia cell line (THP-1) and the human T lymphoma (Jurkat cell line), but not on normal human peripheral blood lymphocytes, and also induced cell cycle arrest at G1 phase by inhibiting the expression of cyclin D protein. The latter was associated with anti-inflammatory activities of the peptide in inhibiting NF-κB activation through inhibition of IkBα phosphorylation, degradation, and p65 nuclear translocation. The SVCIII fraction probably contains the BmK analgesic peptide, BmK AGAP. AGAP, which belongs to a group of long-chain scorpion peptides and has a molecular mass of 7142 Da with 66 amino acid residues, binds to VGSC and has been purified and tested.
### Table 1 In vitro Anticancer Effects of SV or Peptides on Human Cancer Cells

<table>
<thead>
<tr>
<th>Species</th>
<th>Venom/Peptide</th>
<th>Action</th>
<th>Target</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Androctonus</em></td>
<td>Whole venom</td>
<td>Inhibits proliferation of human neuroblastoma (SH-SYSY) and human breast (MCF-7) cancer cell lines</td>
<td>Induces apoptosis through increasing nitric oxide production, caspase-3 activity and depolarizing mitochondrial membrane and arrests S phase</td>
<td>35</td>
</tr>
<tr>
<td><em>crassicauda</em></td>
<td>Non-disulphide-bridged peptides (ND8P), AcrAP1 and AcrAP2, and their high cationic analogues (HC-AcrAP) (0.001-100 µM)</td>
<td>HC-AcrAP analogues inhibit the proliferation of human lung adenocarcinoma (H460), breast carcinoma (MB435s) breast tumorigenic (MCF-7) and prostate carcinoma (PC-3) cell lines (IC50 2-3.6 µM)</td>
<td>Probably inducing cell lysis</td>
<td>68</td>
</tr>
<tr>
<td><em>bicolor</em></td>
<td>Whole venom (500-1000 µg/ml)</td>
<td>Inhibits proliferation of human breast (MDA-MB-231), and colorectal (HCT-8) cancer cells</td>
<td>Induces apoptosis more than necrotic death, and arrests cells at G0/G1 phase, upregulate p53, downregulate Bcl-xL</td>
<td>29-31</td>
</tr>
<tr>
<td><em>Androctonus</em></td>
<td>Whole venom (20-100 µg/ml)</td>
<td>Inhibits cell motility, and prevents colony mitosis of human breast, (MDA-MB-231), and colorectal (HCT-8 and HCT116) cancer cells</td>
<td>Two mechanisms proposed are i) a decrease in the expression of Mmps, and ii) a reduction in the phosphorylation levels of FAK, which is involved in cell migration and invasion.</td>
<td>31</td>
</tr>
<tr>
<td><em>crassicauda</em></td>
<td>Gonearrestide peptide (18 aa, 2192 Da)</td>
<td>2-200 µM</td>
<td>Inhibits the proliferation of human colon cancer cell line HCT116 in dose-dependent</td>
<td>36</td>
</tr>
<tr>
<td><em>Androctonus</em></td>
<td>BmK 70-80kDa</td>
<td>Inhibits proliferation of human acute monocytic leukemia cell line (THP-1) and the human T lymphoma (Jurkat cell line) and induces cell cycle arrest at G1 phase</td>
<td>Inhibits NF-kB activation through inhibition of IkB phosphorylation, degradation and p65 nuclear translocation</td>
<td>44</td>
</tr>
<tr>
<td><em>bicolor</em></td>
<td>SVCIII (1-50 µg/ml)</td>
<td></td>
<td>Enhances expression of apoptotic gene, Bax, reduces anti-apoptotic, Bcl-2, expression, and arrests cancer cells at G1/S</td>
<td>28</td>
</tr>
<tr>
<td><em>Karsch</em></td>
<td>BmK AGAP 71.42KDa with 66 aa (5-60 µM) (IC50 = 40µM for MCF-7 and 50µM for MDA-MB-231 cells)</td>
<td>Inhibits proliferation of DU145 human prostate cancer cell line</td>
<td>Reduces gene and protein expression of Oct4, SOX2, Nanog, N-cadherin, Snail and PTX3, and increased the expression of E-cadherin</td>
<td>47</td>
</tr>
<tr>
<td><em>Buthus</em></td>
<td>Iberiotoxin (IbTX) (34 aa, 36.07 kDa)</td>
<td>Inhibits growth and proliferation of human cervical cancer (HeLa) and human ovarian cancer (A2780) cell lines</td>
<td>Blocks calcium-activated potassium channels KCNMA1 (KCa1.1, BK) *Found to be expressed in cervical cancer-hormone dependent</td>
<td>42-43</td>
</tr>
<tr>
<td><em>tumulus</em></td>
<td>Margatoxin (MgTX, 39 aa, 41.92 kDa)</td>
<td>Inhibits proliferation of human lung adenocarcinoma cells and decreases tumor volume in vivo following intra-tumoral injection</td>
<td>Blocks Kv1.3, and increases expression level of p21Waf1/Cip1 and decreases the expression level of Cdk4 and cyclin D3.</td>
<td>61</td>
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(Continued)
mainly for its pain management.\(^{39,45,46}\) A very recent study by Kampo et al.\(^ {47}\) demonstrated in detail AGAP mechanism of action against two human breast cancer cell lines (Tables 1 and 2). BmK AGAP at concentrations of 5–60 µM inhibited proliferation, sphere formation, colony formation, stemness, and epithelial-mesenchymal transition (EMT) of human breast MCF-7 and MDA-MB-231 cell lines. These phenomena were related to: a) reduction in expression of specific genes and proteins related to cancer cells proliferation (PTX3), stemness (PTX3, Oct4, SOX2, Nanog), EMT (PTX3, N-cadherin, Snail) and b) increase in the expression of E-cadherin (EMT) as well. Furthermore, one of the VGSC subunits, Nav 1.5, which is overexpressed in breast cancer and associated with tumor progression,\(^ {48}\) has been shown to mediate BmK AGAP regulatory action on PTX3 expression/activation and thus reducing the activation of NF-κB and tumor necrosis factor (TNF)-α.\(^ {47}\) Following intraperitoneal

<table>
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<th>Table 1 (Continued).</th>
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<tr>
<td><strong>Heterometrus bengalensis</strong></td>
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<td><strong>Leirus quinquestriatus</strong></td>
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<td><strong>Odontobuthus doriae</strong></td>
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<td><strong>Rhopalurus juncus</strong></td>
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<td><strong>Tityus discrepans</strong></td>
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<td><strong>Tityus serrulatus</strong></td>
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administration of 0.5 or 1 mg/kg of rBmK AGAP into mice bearing subcutaneous xenograft of a human breast MCF-7 or MDA-MB-231 cell lines, tumor growth (volume and weight) was significantly reduced at both doses in comparison to PBS-treated mice (Table 2). These changes were accompanied with a decrease in PTX3, N-cadherin, Snail-1, Oct4, Sox2, β-catenin, pGSK3-β, Nav 1.5, and p65/NF-κB expression and an increase in E-cadherin and GSK3-β in the excised tumors from mice treated with rBmK AGAP.47 In the same study, the authors proved that PTX3 is highly expressed in breast cancer and its expression is associated with cancer stemness and EMT, and BmK AGAP downregulated PTX3 expression via inhibiting or binding to Nav1.5. This inhibition or binding resulted in a downstream reduction of NF-κB and -Wnt/beta-catenin signaling pathway and inflammatory cytokines production. These results demonstrate the potential of BmK AGAP in treating inflammation-induced cancers.

### Angiogenesis Inhibition

Angiogenesis, formation of new blood vessels, is a characteristic of solid cancer cells. Cancer cells overexpress growth factors, vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF), that drive the formation of new blood vessels. SV and their peptides have shown to inhibit the expression of VEGF. For instance, intraperitoneal administration of SV from *Androctonus amoreuxi* has resulted in downregulating the expression of VEGF in the tumor tissue and this was accompanied with a downregulation of the proliferative marker Ki-67 and reduction in the solid tumor size.47 Also, topical administration of *Leiurus quinquestriatus* SV reduced skin carcinogenesis incidence in mice, which was associated with regulating several protein expressions; one of them was VEGF.

Chlorotoxin (CITx), which was originally isolated from *Leiurus quinquestriatus*, is a 36 amino acid in length and has been found to bind glioma cells but not healthy glial brain cells and inhibits CT fluxes across glial membranes.49-51 Similarly, the synthetic peptide of CITx, TM601, was found to be selective to glioma cells but not to non-transformed neurons, astrocytes, or fibroblasts.52 Although TM601 binds to glioma cells, it is non-toxic to cancer cells. However, it has been shown to block angiogenesis in vitro and in vivo.8,53 TM601 treatment of human tumors (SK-Mel melanoma, PC-3 prostate, and U87-MG glioblastoma) grown on the chick chorioallantoic membrane decreased tumor vascularization in

| Table 2 In vivo Anticancer Effects of SV or Peptides on Human Cancer Cells |
|----------------|----------------|----------------|----------------|----------------|
| **Species**    | **Venom/Peptide** | **Action**        | **Target**               | **Ref.** |
| *Androctonus*  | Whole venom     | Reduced Ehrlich ascites carcinoma and solid Ehrlich tumor size, and increased survival of mice-bearing intraperitoneal tumor | Downregulates expression of Ki-67 and VEGF and increased expression of caspase-3 | 37   |
| *Androctonus*  | Gonearrestide peptide (18 aa, 2192 Da) 50 and 100 µM peritumoral injection for 2 weeks | Reduced tumor growth of human colon cancer cell line HCT116 in dose-dependent | Modulates cell cycle checkpoint proteins (down-regulate CDK4, and upregulate cyclin D3, p21, p27) | 36   |
| *Buthus*       | BmK AGAP 71.42KDa with 66 aa (0.5 and 1 mg/kg) injected i.p. for 20 days at 48 h intervals | Reduced tumor growth of MDA-MB231 cells in mice | Reduces gene and protein expression in ex vivo tumors of Oct4, SOX2, Nanog, N-cadherin, Snail and PTX3, and increased the expression of E-cadherin | 47   |
| *Leiurus*      | Whole venom 17.5, 35, 52.5 µg topical twice a week for 16 weeks | Decreased skin carcinogenesis incidence in mice | Downregulates expression of Ki-67, NF-κB, Cox-2, Bcl-2, VEGF and proinflammatory cytokines (TNF-α and IL-6) | 38   |
| *Leiurus*      | TAM-601 (synthetic CITx) at 10 µg | Blocks angiogenesis in chick chorioallantoic membrane growing human tumors | Inhibits VEGF, PDF, TNF-α action on vascularization and blocks MMP-2 activity | 54   |
|                | TAM-601 at 2, 10, or 100 mg/ kg (i.v.), 3x/week for 2 weeks | Reduces microvessel count in mice using Matrigel plug assay |                           |                        |
Invasion and Metastasis Inhibition

SV Peptides, Ion Channels and Immunomodulation

In addition, TM601 has shown to reduce microvessel density.

Similarly, BmK fraction of AGAP, which binds to the subunit of AGAP (Nav 1.5), not only inhibited in vitro and in vivo proliferation, stemness and sphere formation, colony formation, epithelial-mesenchymal transition (EMT), but also migration and invasion of human breast MCF-7 and MDA-MB-231 cells.

Mikaelian et al. 

The authors proposed that SV might be used as anticancer agents. 

Invasion and Metastasis Inhibition

SV isolated from either Androctonus crassicauda or Leiurus quinquestriatus has been found to interrupt metastasis of breast and colorectal cancer cell lines. 

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Therefore, SV peptides play a significant immunomodulatory action on immune cells and cytokine expression that repolarize inflammation-induce cancer processes. Such immunomodulatory action is an essential pathway to regulate cancer development and growth.

**Polarization of SV Venom**

In late 2008, Dr. Mikaelian filed a patent on polarizing diluted SV solution that makes the venom more stable, results-consistent, and have more efficacy in regards improving immune response, treating and preventing cancer as well as relieving pain and reducing the adverse events of chemotherapy and radiation. The polarization step involves placing the SV solution in an electromagnetic chamber of the MRT-06 polarization machine for 5 hrs, where a monopole field is created by an AC power source. In the patent, the author showed the significance of the polarized *Rhopalurus juneus*, a species of *Buthidae*, venom on reducing cancer cell proliferation, tumor size in vivo in a dose-dependent manner, as well as pain.

**Rhopalurus princeps (Rp) Venom**

**Anticancer Effects of Rp Venom**

Following the patent, several studies have been performed on a different species of *Buthidae* family, *Rhopalurus princeps* (Rp) venom and have been tested in vitro on different human cancer cell lines as well as on normal cells (unpublished data). For instance, Rp venom has been tested on the human prostate cancer cell line, BxPC3, and found that polarized Rp venom significantly inhibited BxPC3 proliferation in a concentration- (≥500 µg/mL) and time-dependent (≥48 hr) manner. In other cell lines, polarized Rp SV has eradicated in vitro human ovarian (OVCAR3 and IGR-OV1) and bladder (SCaBER) cell lines in a concentration-dependent manner (Figure 2). On the other hand, high concentrations of the Rp venom did not exhibit cytotoxicity on dermal fibroblasts or normal ARPE-19 cell line.

In apoptosis assay, Rp venom induced apoptosis in the BxPC3 cell line at very low concentrations (Figure 3A). Furthermore, the polarization of Rp venom significantly enhanced the incidence of apoptosis in BxPC3 (Figure 3B and C). However, adding AuNP, a delivery agent, to non-polarized Rp venom increased the apoptotic activity but did not significantly change the percent apoptosis to the polarized venom (Figure 3B and C), indicating that polarization of SV has a similar impact to AuNP on Rp venom-inducing apoptosis. These results suggest that anticancer activity of Rp venom is mainly by inducing apoptosis rather than inhibiting metabolic pathways of cancer cells and secondly polarization of Rp venom induces higher apoptotic effects on cancer cells.

**Structural Analysis of Rp Venom and in silico Studies**

Several studies have been performed to characterize Rp venom. Mass Spectrometry Analysis (MALDI-TOF) revealed 176 molecular mass fragments ranging from ~500–32,000 Da

![Figure 2](https://www.dovepress.com/figure2-survival-curves-of-three-human-cancer-cell-lines-bladder-squamous-cell-carcinoma-cell-line-scaber-obtained-from-nci.jpg)
The six disulfide bridges, C1(2)-C4(19), C2(5)-C6(28), C3(16)-C7(33), and C4(20)-C8(35), and are cross-linking the alpha-helix to the beta-sheets. The fourth disulfide bridge (C12-C4(19) links the small N-terminal beta-strand to the rest of the molecule (Figure 4).

Furthermore, other ion channel peptides inhibitors have been found in the Rp venom. The calcium voltage-gated peptide inhibitor consists of 34 amino acids, including six cysteines and three disulfide bonds. This inhibitor belongs to the inhibitory cysteine knot structural family that includes a compact disulfide-bonded core from which four loops emerge. The disulfide bonds are as follows: C1(5)-C4(20), C2(12)-C5(26), C3(19)-C6(33). This peptide contains a 2-stranded antiparallel beta-sheets (residues 26–27 and 32–33) as the only secondary structures. Similarly, the potassium channel peptide inhibitor has 31 amino acids in including six cysteines and three disulfide bonds (Figure 4). The structure consists of a double-stranded antiparallel β-sheet (sequences 19–23 and 26–30) and a single helix (sequences 4–15) covering one face of the α-sheet. The cysteine side chains connecting the β-sheet and the helix form the core of the molecule. The disulfide bonds are as follows: C1(3)-C4(22), C2(8)-C5(27), C3(12)-C6(29) (Figure 4). Finally, sodium channel peptide inhibitor is also present in Rp venom and consists of 76 amino acids in including twelve cysteines and six disulfide bonds. The six disulfide bonds are as follows: C1(4)-C4(19), C2(11)-C5(24), C3(18)-C6(34), C7(41)-C10(56), C8(48)-C11(61), C9(55)-C12(72). This peptide inhibitor contains 4-stranded antiparallel beta-sheets (residues 22–24; 34–36; 59–62; and 71–74) as the only secondary structures.

**Concluding Remarks**

In the present review, we show the pleiotropic effect of SV and their peptides in a) inhibiting the proliferative characteristics of cancer cells by mainly inducing apoptosis without affecting healthy cells such as lymphocytes proliferative capabilities, b) interrupting the invasion and metastatic capabilities of cancer cells, c) inhibiting angiogenesis and d) modulating the immune cell function by lowering the inflammatory cascade that occurs in some cancer types. Thus, these encouraging results signify the potential of SV peptides in targeting multiple events in cancer cells development and metastasis.

**Table 3** MALDI-TOF Analysis of *Rhopalus princeps* Venom

<table>
<thead>
<tr>
<th>Mass Range (m/z)</th>
<th>Number of Fragments</th>
<th>Daltons</th>
</tr>
</thead>
<tbody>
<tr>
<td>480–5000</td>
<td>30</td>
<td>494.5–4247.6</td>
</tr>
<tr>
<td>600–25,000</td>
<td>75</td>
<td>819.3–17,596.8</td>
</tr>
<tr>
<td>800–60,000</td>
<td>71</td>
<td>1979.1–31,840.8</td>
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(Table 3). Following BLAST analysis, one of the peptides in Rp venom, termed (Rp-CITx), resembles in sequence and amino acid length the chlorotoxin peptide from Leiurus quinquestriatus hebraeus – the Death Stalker scorpion (Lqq-CITx) and others (Table 4). Rp-CITx shares the highest sequence identity (97%) with Lqq-CITx. The in silico study revealed that Rp-CITx has 4-stranded antiparallel beta-sheets (residues 22–24; 34–36; 59–62; and 71–74) with four disulfide bridges, C1(2)-C4(19), C2(5)-C6(28), C3(16)-C7(33), and C4(20)-C8(35), and are cross-linking the alpha-helix to the beta-sheets. The cysteine side chains connecting the β-sheet and the helix form the core of the molecule. The disulfide bonds are as follows: C1(5)-C4(20), C2(12)-C5(26), C3(19)-C6(33) (Figure 4). Finally, sodium channel peptide inhibitor is also present in Rp venom and consists of 76 amino acids in including twelve cysteines and six disulfide bonds. The six disulfide bonds are as follows: C1(4)-C4(19), C2(11)-C5(24), C3(18)-C6(34), C7(41)-C10(56), C8(48)-C11(61), C9(55)-C12(72). This peptide inhibitor contains 4-stranded antiparallel beta-sheets (residues 22–24; 34–36; 59–62; and 71–74) as the only secondary structures.
However, it has to be addressed that the concentrations used to show an inhibitory effect for some whole SV were high (IC\textsubscript{50} > 500 µg/mL), whereas the IC\textsubscript{50} concentrations significantly became low when specific peptides isolated, or recombinant type were used. Interestingly, some recombinant peptides became more anti-proliferatively potent when more cationic amino acids were introduced in the peptide (Table 1).\textsuperscript{71–73} The latter phenomenon could be initiated by increasing the electrostatic interaction between cationic peptides and the negative membrane charges of tumor cells. The high negative charge of tumor cells is mainly due to the presence of phosphatidylserine, a negatively charged amino acid, on the outer surface of the plasma membrane of tumor cells. Similarly, the polarization of Rp venom showed better anticancer potential in increasing the apoptosis in cancer cells.

A recent review by Marshall & Djamgoz\textsuperscript{5} pointed out several novel immunotherapy co-targets in pre-clinical development, and one of these targets is ion channels. Since ion channels dysregulation are a factor in cancer development, targeting them would be a unique tool multi-faceted advantages for T cell-based immunotherapy.\textsuperscript{5} Therefore, we believe that the immunomodulatory effects of SV and their specific peptides would benefit the immune-oncology research programs mainly with the checkpoint inhibitors such as PD-1 and CTLA-4 blockade in reducing tumor microenvironment immunosuppression, immune-checkpoint resistance and enhancing cancer patient response.

Before clinical efficacy is established, non-clinical safety is an evident marker of the success of any pharmaceutical agent in translation medicine. Synthetic CITx peptide (tozuleristide, or BLZ-100) has shown to be safe and has not exhibit any adverse events in rats and non-human primates following a single intravenous dose of 28 mg/kg and 20 mg/kg, respectively.\textsuperscript{74} Furthermore, in Phase 1 clinical trial, no adverse event was observed following an intravenous dose of up to 30 mg.\textsuperscript{75} These targeted and promising results of SV open the venue for full non-clinical safety profiles to be performed before clinical translation into cancer therapy.

**Disclosure**

AGM is the Chief Executive Officer of Medolife Corporation and owns a patent on SV polarization. ET and XMZ are scientists working at Medolife Corporation. KZM is employed by OncoTherapeutica, Inc. The authors report no other conflicts of interest in this work.
Three dimensional structures of chloride, calcium, potassium and sodium ion channel inhibitors present in *Rhopalurus princeps* venom (Rp) showing the α-helix, β-sheets and the disulphide bridges in each inhibitor.

**Figure 4**

### References


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