REVIEW
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Scorpion Venom and Its Different Peptides Aid in Treatment Focusing on Cancer Disease with the Mechanism of Action

ABSTRACT

Animal venoms, especially those from scorpions, have garnered interest for their potential therapeutic uses. Scorpion venom is a complex mixture of bioactive compounds, including neurotoxins, enzymes, enzyme inhibitors, and peptides. These peptides have shown promise in pharmacological applications due to their high selectivity and relatively safe mechanisms of action. In this review, we focused on cancer, one of the leading causes of death worldwide, and explored the potential of scorpion venom peptides as promising therapeutic agents in cancer treatment. Despite significant advancements in cancer therapy, many cases remain reliant on palliative care, particularly for treatment-resistant cancer types. Scorpion venom peptides, with their specific and targeted mechanisms of action, exhibit potential as novel anticancer agents. These peptides have demonstrated the ability to selectively target cancer cells, inhibit tumor proliferation, and modulate immune responses, positioning them as valuable candidates for the development of more effective therapies. Previous studies have highlighted that certain venom-derived peptides can suppress tumor growth and metastasis, underscoring their potential to enhance clinical outcomes. Consequently, scorpion venom offers new perspectives and avenues for developing next-generation cancer treatments.

Keywords: Scorpion venom peptide, toxins, anti-cancer, cancer therapy

INTRODUCTION

Venomous animals have long intrigued humans, influencing various cultural, ecological, and economic aspects of life.[1,2] Venom, a successful evolutionary adaptation, has independently evolved over 100 times across major animal lineages, primarily for defense or predation. In almost all natural ecosystems, venomous species play pivotal roles in ecological networks, utilizing venom in interspecific interactions such as predation (e.g., spiders, scorpions, centipedes, and snakes) or defense (e.g., bees, sea urchins, and fish).[3,4] The co-evolutionary arms race has refined venom components, making them highly potent disruptors of physiological systems.

The co-evolutionary processes that drive the diversity and specificity of venoms are still not fully understood, and broader comparative studies are needed to unravel these complexities. The remarkable target selectivity of many venom compounds has long intrigued researchers, fueling interest in their potential applications in both applied and translational research. Over the past several decades, molecules derived from select species, including cone snails, snakes, spiders, and scorpions, have been extensively studied to explore their bioactivity. These toxins are now being utilized across a wide range of translational fields, including drug development, sustainable bioinsecticides, and clinical biomarkers for diagnostics.[1,2]

Animal venoms are complex mixtures of bioactive compounds that exhibit high affinity for various targets in cells and tissues. Scorpion venoms, along with their isolated peptides, have demonstrated significant effects on cancer cells (Figure 1) through 4 probable mechanisms: i) activation of cell cycle arrest, growth inhibition, and apoptosis; ii) inhibition of angiogenesis; iii) inhibition of invasion and metastasis; and iv) blockage of specific transmembrane channels.

Radwa Abdallnasser Amen[1]
Gamal Eldein Fathy Abd-Ellatef[2]

[1]*Department of Biotechnology, Cairo University, Faculty of Science, Cairo, Egypt*
[2]*Department of Therapeutic Chemistry, Pharmaceutical and Drug Industries Research Institute, National Research Centre, Cairo, Egypt*

Corresponding author:
Gamal Eldein Fathy Abd-Ellatef
✉ ge.fathy@nrc.sci.eg

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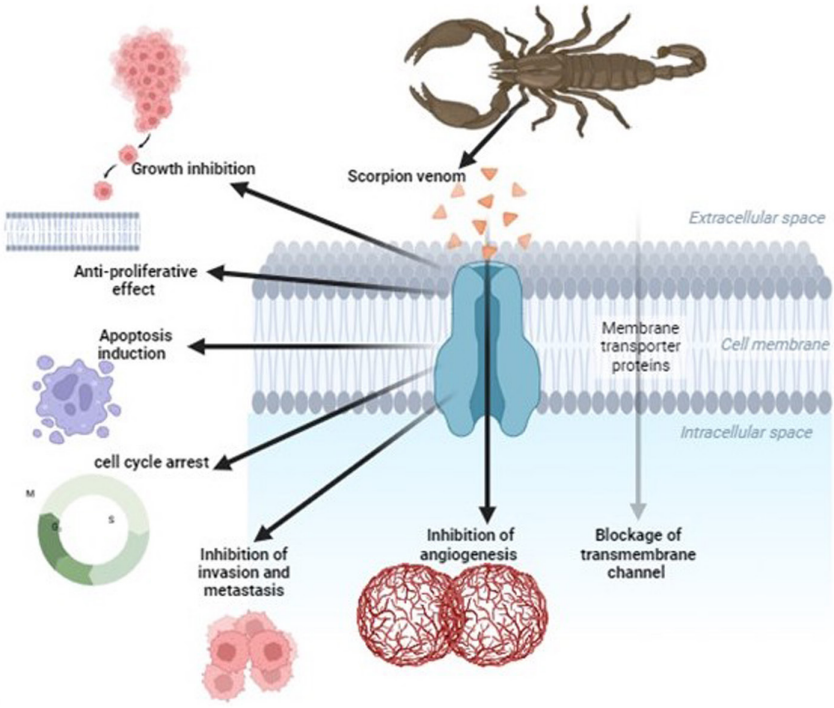

Figure 1. Scorpion venoms' significant impacts on cancer cells.[1]

Tumor biology is inherently complex, involving numerous interconnected pathways, as evidenced by cancer's putative hallmarks. This complexity presents multiple opportunities for pharmacological intervention. Molecules capable of targeting multiple proteins or signaling pathways, such as those found in animal venoms, hold promise as potent anticancer agents.[5]

Various scorpion venoms have substantial therapeutic potential. They are high in neurotoxins, enzymes, enzyme inhibitors, histamine, lipids, and various salts, from which peptides show significant promise in treating a variety of disorders. Non-disulfide-bonded peptides govern several biological activities, including bradykinin potentiation, hemolysis, anticancer, antimicrobial, and anti-inflammatory potential. It is used in the treatment of cancer, cardiovascular illness, diabetes, acquired immunodeficiency syndrome, apoplexy, influenza H5N1, paralysis, epilepsy, malaria, measles, severe combined immunodeficiency, fever blisters, and diabetes seems appealing. Scorpion venom contains over 100 000 bioactive compounds.[6]

It is thought that scorpions, or pieces of them, or their venoms, are useful in the treatment of many illnesses, including cancer. It is now evident that scorpion venoms contain a wide range of other peptides in addition to poisons thanks to the advancement of contemporary technologies for the extensive examination and characterization of venom components. Numerous physiologically active peptides have demonstrated their value in the creation of drugs intended to treat a broad spectrum of grave illnesses.[7] A scorpion sting can cause everything from minor health issues to life-threatening ones, even death. The degree of envenomation is determined by the presence of neurotoxins in scorpion venom.[8] They can cause autonomic excitement by blocking or altering the function of the ion channels they are targeting in excitable cells. Significant endogenous catecholamine release is caused by scorpion venom. A series of physiological events, including arterial hypertension or hypotension, tachycardia or bradycardia, arrhythmia, unconsciousness, pulmonary edema, heart failure, and death, can result from the combination of sympathetic stimulation and catecholamine release in plasma.[9] A complex mixture of proteins and peptides, scorpion venom can have pharmacological and toxicological effects. Apart from pharmacological properties including antipyretic, analgesic, and anti-inflammatory properties.[10] Peptides are regarded as promising medicines due to their great selectivity and relatively safe mode of action. Peptides that have been separated into individual molecules can be useful drugs when used in large enough numbers. These little peptides are the most researched parts of scorpion venom due to their variety and range of medicinal uses. Based on their structural characteristics, peptides have been divided into 3 superfamilies' calcins, peptides with cysteine-stabilized patterns, and non-disulfide bridged peptides (NDBPs).[11] This review focused on addressing different types of peptides and their treatment properties, the anticancer properties, and the different mechanisms of scorpion venoms.

VARIOUS SCORPION SPECIES IN CANCER TREATMENT

The blue (or red) scorpion *Rhopalurus junceus* is well-known for its antineoplastic qualities in the Dominican Islands and Cuba. Its venom relieves pain and keeps cancer patient's alert. It has a protein that can stop the growth and multiplication of cancer cells.[12,13] The venom of the Red or Blue Scorpion *R. junceus* improves vitality and lessens pain in cancer patients. This venom extracted from blue scorpions is analgesic, anti-inflammatory, and anti-cancer.[14] The venom of *Tityus discrepans* scorpions contains peptides, such as neopladine, that cause substantial abnormalities and cause human breast cancer cells to undergo apoptosis.[15] Proteolytic enzymes including lactase dehydrogenase, a cytotoxic and apoptotic agent that can lower cell viability by activating caspase-3 and depolarizing mitochondria, are found in the venom of *Odontobuthus doriae*. Proteolytic and gelatinolytic proteases from the scorpion *Mesobuthus gibbosus* are effective against human lung cancer cell lines.[16] The venom of *O. doriae* induces apoptosis in human breast cancer cells by halting the S phase and elevating reactive nitrogen intermediates.[17] Peptides that decrease tumor size are discovered in *Centruroides margaritatus* venom.[18] The venom of scorpions contains many substances that can change the growth, proliferation, and cell cycle. Cancer has been treated with *R. junceus* in Cuban traditional medicine. *Heterometrus bengalensis*, the Indian black scorpion, has venom that can inhibit the growth of K562 and U937 cells while also displaying characteristics of apoptosis, including chromatin condensation, DNA damage, and membrane blebbing. The venom of the Deathstalker scorpion, *Leiurus quinquestriatus*, contains 36 amino acid peptides that inhibit chloride channels.[19,20] In chronic myelogenous and bengaline cells, *H. bengalensis* exhibits apoptogenic and antiproliferative properties. The venom of the Chinese red scorpion *Buthus martensia* provides antineoplastic medicines without any dangerous side effects, as well as hyaluronidase (BmHYA1), which is responsible for metastasis and reduces the proliferation of breast cancer.[21,22]

COMPONENTS AND PEPTIDES OF SCORPION VENOM

Scorpion venoms consist of intricate blends of peptide, mucoproteins, enzymes, nucleotides, lipids, free amino acids, amines, inorganic salts, heterocyclic compounds, and potentially other unidentified substances. The parts of scorpion venom that have drawn the greatest interest are called toxins. This is due to both their clinical importance as neurotoxins and their action on ion channels pharmacologically. These are peptides with a severely limited structure and disulfide bridges. The most significant effects on mammals, including humans, are triggered by toxins that act on sodium channels. They fall into 2 categories: toxins, which prevent the voltage-gated Na^+ channel from becoming inactivated, and toxins, which raise the negative potential at which the channels open.[23] At modest doses, toxins significantly depolarize the cell membrane, which is followed by a decrease in excitability. Increased dosages result in cardiac arrhythmias and paralysis by extending excitable cells' action potential.[24] Toxins act to produce myoclonic or spastic muscle responses.[25] Other recognized toxins from scorpions affect the calcium, chlorine, and potassium channels. Despite the fact that they may work in concert to exacerbate clinical symptoms, their importance in human envenomation seems to be secondary. While the most recognized impacts of scorpion venom toxins, or their related components, are on cells, tissues, and organisms, some toxins have been found to have properties that could be valuable in developing pharmaceutical drugs. Among them are antimicrobial, antimalarial, immunosuppressive, and anticancer effects. Non-disulfide bridged peptides are among the other components found in scorpion venom.[26]

The venom of scorpions contains a significant amount of the NDBP family. More than one-third of the molecular weights found in mass-fingerprint investigations utilizing whole venoms are attributed to low-molecular-weight peptides.[27] Up to the last 10 years, the discovery rate of NDBPs trailed behind the body of knowledge on toxins due to the predominant focus of research on greater molecular weight peptides in dangerous venom fractions. Researchers' attention has been sparked by the finding that NDBPs can display significant biological roles. This has increased the amount of information on these small peptides, along with the availability of molecular biology techniques like heterologous expression, cDNA library generation, and most recently RNA-Seq.[28] Only a few dozen NDBPs have been found thus far, even though hundreds of toxins have been recovered and described from the venoms of scorpions.[29]

The small 13-56 amino acid peptides that make up the NDBP venom have incredibly diverse sequences. Most of them have exceptional structural flexibility and are cationic. They exist in random coil conformations in aqueous solutions, but they quickly assume amphipathic helical forms in circumstances that mimic membranes, such as 50%-60% aqueous trifluoroethanol. The negatively charged lipid head groups of biological membranes are easily manipulated by positively charged NDBPs. Membrane adhesion triggers the insertion of hydrophobic residues into the membrane and the formation of the amphipathic helix, which in turn causes their activity. They have a wide range of biological targets because there isn't a single molecular target. Numerous NDBPs perform multiple tasks that are not dependent on the target cells. This is in stark contrast to how toxins work, which involves poisons targeting certain receptors (ion channels) on particular cellular targets.[30] The identified NDBPs from scorpion venom have demonstrated anticancer, antifungal, antibacterial, antiviral, antimalarial, cytolytic, immunomodulating, and bradykinin-potentiating properties.[29] There are a diversity of

peptides found in scorpion venom and their potential uses in medical research and treatment as shown in Table 1.

SCORPION PEPTIDES EFFECT AGAINST CANCER

Anticancer peptides are an important source for the development of tumor-targeting drugs, which could enable a more thorough examination of the process by which cancer develops. Certain minuscule chemicals exhibit efficient tissue penetration and uptake by malignant cells. They could function as carriers for drugs with lower selectivity or bioavailability, work in concert with current chemotherapeutics, or directly affect tumors through intrinsic activity. A potential source of physiologically active substances with anticancer properties is the venom of scorpions.[42] Previous in vitro and in vivo studies have demonstrated the anticancer effects of several scorpion proteins and peptides; one of these compounds has even advanced to phase I and phase II clinical trials.[43]

Chlorotoxin (ClTx, UniprotKB P45639) is a peptide derived from the venom of the scorpion *L. quinquestriatus*, consisting of 36 amino acids and 4 disulfide bonds. It possesses 1 radioiodinable tyrosine residue. The peptide is classified as a knottin by nuclear magnetic resonance spectroscopy, which shows that its solution structure is made up of a short, 3-stranded antiparallel sheet that is cross-linked to a helix by 3 disulfide bonds. A fourth disulfide bond joins the little N-terminal beta strand to the rest of the molecule. The initial research discovered that ClTx can inhibit small-conductance chloride channels formed by epithelial cells.[44] It was then shown to bind to chloride channels that are only expressed on human glioma and astrocytoma cells. It has been shown that[45] ClTx binds to metalloprotease-2 (MMP-2) to block these chloride channels.[46]

A cDNA sequence that encodes a short-chain toxin with 4 disulfide bridges, known as Bm-12 or chlorotoxin-like peptide (BmKCT), was previously cloned from the venom gland of *Buthus martensii Karsch* (BmK). In 68% of cases, it matched the chlorotoxin that was isolated from *L. quinquestriatus*. Chloride currents in solitary glioma cells were decreased by recombinant BmKCT (rBmKCT) (U2251).[47] In addition, the toxin inhibited the proliferation of human glioma (SHG-44) cells, which did not damage normal astrocytes. Following intraperitoneal administration of rBmKCT to normal mice, histological analysis demonstrated damage to the heart, legs, and brain. C6 glioma cells were preferentially linked with 131I-BmKCT in vitro, which inhibited cell proliferation.[48] The combination therapy of BmKCT and lithium chloride (LiCl) decreased metalloproteinase-2 activity, as well as the motility, invasion, and proliferation of C6 glioma cells.[49] To transfer the BmKCT gene to C6 glioma cells, a recombinant replication-defective adenovirus (Ad) delivery method was recently created.[50]

From a cDNA library made from the venom gland of *Buthus martensii Karsch*, the sequence of the antitumor-analgesic peptide (BmK AGAP) was found, and it had a strong identity to the sodium channel modulator-toxins.[51] Subsequent research revealed the analgesic and antitumoral qualities of BmK AGAP. Buthus martensii Karsch antitumor-analgesic peptide's analgesic effect was proven in hot-plate and mouse twisting tests.[52] Ehrlich and S-180 fibrosarcoma were used to illustrate the in vivo anticancer activity. Recombinant rBmK AGAP (rAGAP) inhibited the development and proliferation of glioma cells by causing

Table 1. Examples of Some Scorpion Venom Peptides and Their Potential Use and Function in Medical Research and Treatment

Peptide Name	Scorpion Species	Function/Use	Medical Applications
Chlorotoxin	*L. quinquestriatus (Deathstalker Scorpion)*	Binds to chloride channels	Tumor imaging and targeting (e.g., gliomas).[31]
Maurotoxin	*Scorpio maurus palmatus*	Potassium channel blocker	Potential treatment for autoimmune diseases.[32]
Iberiotoxin	*Buthus tamulus*	Potassium channel blocker	Hypertension, a potential treatment for autoimmune diseases.[33]
Margatoxin	*C. margaritatus*	Potassium channel blocker	Immune response modulation, a potential treatment for autoimmune diseases.[34]
Scyllatoxin	*L. quinquestriatus (Deathstalker Scorpion)*	Calcium channel blocker	Research in neurophysiology.[35]
Meucin-24 and Meucin-25	*Mesobuthus eupeus*	Antibacterial peptides	Potential antibiotic alternatives.[36]
TsAP-1 and TsAP-2	*Tityus serrulatus*	Antimicrobial peptides	Potential antibiotic alternatives.[37]
Androctonin	*Androctonus australis*	Antimicrobial peptide	Potential antibiotic alternative. [38]
Imperatoxin I	*Pandinus imperator*	Calcium release channel modulator	Research in muscle physiology.[39]
Stigmatoxin	*Tityus stigmurus*	Calcium release channel modulator	Research in neurophysiology, potential therapeutic applications.[40]
Smp24, Smp43	*Maurus Palmatus*	Calcium release channel modulator	Potential antibiotic alternative/ anticancer effect against cancer cells.[41]

cell cycle arrest in the G1 phase and blocking the action of proteins involved in growth and cell cycle regulation.[53] Recently, a new peptide exhibiting dual-function analgesic and anticancer activity was found in the venom of the same scorpion species. This peptide bore 94% similarity to BmK AGAP. BmK AGAP-SYPU2 is a fascinating peptide found in scorpion venom that may have analgesic effects.[54]

Intermediate-Small Conductance Potassium channel (ISK) which is a slowly activating K^+ channel cloned from smooth muscle and the heart, Maxi-K channels, the delayed rectifier of mouse pancreatic cells, and the n-type current of human T-lymphocytes (Kv1.3 channel) were all unaffected by margaritoxin (MgTx), a 39-amino-acid peptide isolated from the venom of *Celestichthys margaritatus*. By acting on accumulation and a decrease in Cdk4 and cyclin D3, MgTx reduced cell proliferation in the human lung adenocarcinoma A549 cell line, causing an increase in the G1 phase and a decrease in the S phase. This mechanism involves p21Waf1/Cip1, also known as cyclin-dependent kinase inhibitor 1 or CDK-interacting protein 1.[55]

Purified from the venom of *Mesobuthus tumulus*, iberiotoxin (IbTx) is a 37-amino acid peptide that shares 68% sequence identity with charybdotoxin (ChTX). It was previously shown to be a selective blocker of high conductance calcium-activated (maxi-K, also called BK-channel) potassium channels. In human 1321N1 astrocytoma cells, IbTx blocked BK-channels, which stopped K^+-channel-induced proliferation. Because IbTx blocked BK-channels, glioma cells entered the S phase and eventually died. The hormone-insensitive prostate cancer cell line PC-3, which possesses functional overexpression and amplification of the *KCNMA1* gene which encodes the pore-forming subunit of the Maxi-K channel grew less rapidly when treated with IbTx.[56] Calcium-activated potassium channels are blocked by the 37 amino acid residue ChTx that was extracted from the venom of *L. quinquestriatus hebraeus*. Charybdotoxin does not distinguish between K^+ channels that are Ca^{2+}-activated. Human melanoma cell motility and NIH3T3 fibroblast motility were both reduced by ChTx, most likely as a result of inhibiting K^+ channels, which reduced the electrochemical driving force for Ca^{2+} entry.[57]

The whole venom of *H. bengalensis* exhibits antiproliferative and apoptogenic effects on the human leukemic cell lines U937 and K562.[58] Subsequently, it was found that *bengaline* a high molecular weight protein (72 kDa), exhibited anticancer activity against human leukemic cell lines by inducing apoptosis. This process was primarily mediated by the mitochondrial pathway, which involved pro and anti-apoptotic proteins. Notably, normal human lymphocytes were not significantly killed.[59] Previous study discovered that a mechanism other than apoptosis may be involved in *bengalin*-induced cell death, and this mechanism may be autophagic in nature.[60] *Bengalin* also demonstrates cardiotoxicity and neurotoxicity in in vivo testing, as well as anti-osteoporosis action through the restoration of bone minerals.[61] Two amidated peptides with antibacterial and anticancer qualities, TsAP-1 and -2, were extracted from the venom of the Brazilian yellow scorpion *T. serrulatus*. Although they are quite comparable, TsAP-2's higher helical content and hydrophobic moment relative to TsAP-1 explain the variation in antibacterial effectiveness. In previous studies that used the cancer cells H157 (oral squamous carcinoma), H838 (lung adenocarcinoma), MCF-7 (breast carcinoma), PC3 (prostate carcinoma), and U251-MG (glioblastoma) showed that the anticancer cell activity of synthetic analogs with increased cationicity was evaluated.[62]

Neopladines 1 and 2, which are proteins with molecular weights of 29 918 Da and 30 388 Da, respectively were extracted from the venom of *T. discrepans*. They induced the expression of Fas ligand (FasL) and activated Fas signaling on SKBR3 human breast cancer cells to demonstrate apoptotic activity.[15] BmHYA1, a pure hyaluronidase enzyme isolated from *M. martensi Karsh*, has a molecular mass of 48 696 Da and is capable of degrading various hyaluronan fragments. The MDA-MB-231 breast cancer cell line's hyaluronan was depleted by BmHYA1, which led to the downregulation of a CD44 protein variant that is prevalent in cancer cells.[63] It is commonly known that hyaluronan is associated with tumor aggressiveness and is upregulated in breast cancer.[64]

A peptide called maurocalcine (MCa), derived from the *Scorpio maurus palmatus*, contains 33 amino acid residues and binds to the ryanodine receptor (RyR). The first scorpion toxin with a high affinity and specificity for RyR, imperatoxin A (IpTxa) is structurally similar to a portion of the skeletal muscle dihydropyridine receptor's II–III loop which has been suggested to function as an activator of RyR1.[65] Cell-penetrating peptides are a class of peptides that may translocate across the plasma membrane in seconds to minutes, including several peptides that are known to function on RyR there are 4 known scorpion calcins.[66] For instance, in vitro MCa demonstrated efficacy as an intracellular delivery agent for doxorubicin (Dox) in MCF7 and MDA-MB 231 cells, which are cell lines with high- and low-invasive forms of breast cancer, respectively (Dox-sensitive and Dox-resistant). Doxorubicin and MCa together allowed a Dox-resistant cell line to overcome its drug resistance.[67] Numerous MCa analogs with reduced toxicity or shorter durations have been created, and it has been shown that they can force different molecules into the intracellular space.[68]

POTENTIAL ANTICANCER AGENTS FROM SCORPION VENOM

One of the earliest known arthropods in the kingdom Animalia is the scorpion. It is widespread throughout the planet and has existed for over 400 million years. Of the 1700 species of scorpions known to science, just 30 species the Buthidae family are thought to pose a threat to humans.[69] Recently, 21 species and 1 subspecies of scorpions with medical significance have been identified in

Mexico and the United States. In Mexico, *Centruroides* is the most common and plentiful genus.[70] In many countries, there is a serious risk to human health from scorpion stings. Every year, more than 1.5 million scorpion stings are reported globally, with an approximate 3000 cases being fatal.[71] Since ancient times, scorpion venom has been used in traditional medicine in many countries, most notably Africa, Spain, India, Cuba, and China.[72]

Numerous substances, including neurotoxins, cardiotoxins, hemolytic toxins, enzymes, lipids, nucleotides, mucopolysaccharides, and biogenic amines, are found in scorpion venom. The main ingredients of venom are neurotoxins which are low-molecular-weight proteins that cause toxicity by obstructing or changing ion channel function.[73] Ion channels are crucial membrane proteins that allow specific ions to pass through the plasma membrane. The cell's outside and inside are separated by the plasma membrane. Each ion species forms an electrochemical gradient between the cytoplasm and the external medium across the plasma membrane when ionic concentrations inside the cell are kept at considerably different levels than those in the extracellular fluid. It creates an aqueous pore that opens up the ion channels when the protein's structure undergoes a conformational change.[74] Whereas ligand-activated ion channels are activated by the binding of a particular ligand to the ion channel, voltage-activated ion channels are regulated by the membrane potential.[75]

The regulation of cancer cell motility and survival relies on ion transport through channels across the cell membrane. In actuality, fundamental tumor cell processes such cell volume regulation, migration, cell cycle progression, and cell proliferation depend on ion transport across the cell membrane.[76] These mechanisms are necessary for cancer cells to survive and proliferate on different transporters or ion channels, such mitochondrial channels.[77] Numerous H^+ transporters are involved in cell division, tumor growth, migration, and proliferation. These include vacuolar H^+-ATPases, H^+/Cl^- symporters, monocarboxylate transporters, and Na^+-dependent Cl/HCO3 exchangers. Moreover, a number of subcellular compartments, including the nucleus, endoplasmic reticulum, Golgi apparatus, lysosome, and mitochondria, express intracellular ion channels that are crucial for the initiation and/or progression of cancer. The outer or inner mitochondrial membrane is home to the majority of the oncogenic intracellular channels that have been identified.[78]

The voltage-dependent anion channel plays a key role in controlling mitotic death and mediates metabolic crosstalk between the mitochondria and the remainder of the cell. In fact, a sequence of events leading up to the opening of the permeability transition pore including depolarization of the mitochondria, production of reactive oxygen species, release of Ca^{2+} from the mitochondria, and swelling of the mitochondria cause ruptures in the outer membrane of the mitochondria, resulting in the release of intramembranous proteins and elevating this channel to a potential target for cancer treatment. The invasiveness and metastasis of breast cancer are correlated with the expression of the mitochondrial calcium uniporter. The most prevalent potassium channel in T cells,[79] Kv1.3, is a member of the Shaker family. Many types of cancer have altered Kv1.3 expression. Within the inner mitochondrial membrane of tumors including those of the breast, ovarian, bladder, esophagus, testicular, colorectal, kidney, pancreatic, lung, prostate, and leukemia, there is a family of proteins known as the uncoupling protein family that carry electrons. A class of chloride-permeable channel implicated in the development of cancer is called a chloride intracellular channel (CLIC). The most well-researched member of a family of channel proteins that is mostly conserved from Caenorhabditis elegans to humans is chloride intracellular channel 4 (CLIC4). Early in the carcinogenesis process is often when alterations in CLIC4 expression and subcellular localization take place. Chloride intracellular channel 4 is becoming known as a potential biomarker for monitoring the development and recurrence of tumors in a range of human cancers.[80]

One of the largest cation channel families is the transient receptor potential (TRP) channel superfamily. The 28 members of the TRP family are TRPC (canonical), TRPM (melastatin), TRPP (polycystin), TRPV (vanilloid), TRPML (mucolipin), and TRPA (ankyrin-like). Two of them, TRPM8 and TRPC1, have been connected to the onset and spread of cancer. Many tumors overexpress TRPM8, a calcium-permeable protein present in the endoplasmic reticulum membrane.[81] Multiple types of cancer cell lines are inhibited from growing by the venoms of scorpions. However, only a small subset of toxins exhibits anticancer properties through 3 distinct mechanisms: i) blocking a particular ion channel, ii) preventing cancer cells from invasively entering the cell by binding to a particular site in the plasma membrane that is distinct from an ion channel, and iii) triggering intracellular pathways that trigger apoptosis. In addition, they are linked to the emergence of traits unique to cancer, including metastasis, evasion of apoptosis, limitless replicative potential, and insensitivity to anti-growth signals.[82,83]

Scorpion venom has been shown in previous studies to have strong anticancer cell-line effects. At a concentration of 100 g/mL, *Androctonus crassicauda* caused 98% of the HCT-8 cell line (human colorectal adenocarcinoma) to die within 24 hours, demonstrating its anticancer potential. *Androctonus amoreuxi* was shown to exhibit 93% cancer cell death after 24 hours at a concentration of 5.58 g/mL against the PC-3 cell line (human prostate adenocarcinoma), demonstrating its anticancer properties. *A. amoreuxi* showed anticancer effectiveness against the human breast adenocarcinoma MCF-7 cell line at a dosage of 0.61 g/mL, killing 83.7% of cancer cells in a 24 hour. In another study, *A. crassicauda* at 80 g/mL showed anticancer activity against the MDA-MB-231 cell line (human

breast adenocarcinoma), resulting in the death of 24% of cancer cells within 24 hours. At a dosage of 100 g/mL, *A. crassicauda* demonstrated anticancer efficacy against the HCT-116 cell line (human colorectal carcinoma), killing 80% of cancer cells. The human breast adenocarcinoma (MDA-MB-468 cell line) was tested for anticancer activities with *R. junceus* (1 mg/mL), and the results showed that 64.5% of the cancer cells died after 72 hours. In the MDA-MB-231 cell line, *Androctonus bicolour* (100 g/mL) showed tumoricidal action, leading to 63% cancer cell death after 24 hours. In a similar vein, after 24 hours, *L. quinquestriatus* eliminated 63% of MDA-MB-231 cancer cells.[84] With 61.5% of the cancer cells dying after 72 hours, it was previously discovered that *R. junceus* possesses anticancer activities against the human lung carcinoma (A549 cancer cell line). When tested for tumoricidal efficacy against the HeLa cancer cell line, uterine cervix adenocarcinoma, *B. martensii Karsch* produced 50% cancer cell death at a dosage of 34.5 g/mL.[85]

MECHANISM OF ACTION OF SCORPION VENOM ON CANCER CELLS

Biomolecules found in scorpion venom have been proven to influence cancer-related markers. As an up-regulator caspase 3, many mechanisms may have an influence on cancer cells: activates p21; decreases mTOR; decreases vascular endothelial growth factor (VEGF); decreases micro-vessel density; reduces MMP-2; inhibits proliferation; inhibits migration; arrests G1 on cell cycle; decreases MMP-9; blocks K^{2+} channels; suppresses proliferation; induces DNA fragmentation; reduces cell motility and colony formation; arrests cell cycle in S phase; depolarizes mitochondrial membrane; induces FasL expression; induces mitochondrial depolarization; increases p53; and reduces bcl-2 mRNA; angiogenesis should be avoided. Influences and blocks Na^{+} channel, causes hole development and membrane destabilization the mitochondrial death pathway is activated; p27 is upregulated. Scorpion venom causes cell cycle arrest, suppression of growth, and apoptosis. In general, scorpion venoms inhibit angiogenesis, invasion, and metastasis. Scorpion venoms block certain transmembrane channels. Toxin activity on diverse ion channels. Potential against malignant cell proliferation.[86]

Many scorpion venoms and their associated toxins have tumoricidal properties, and one of these properties is apoptosis. Extrinsic and intrinsic pathways are the 2 mechanisms that cause it to happen. By negatively regulating B-cell leukemia/lymphoma 2 (Bcl2), which inhibits its release, and positively regulating Bcl2-associated X (Bax), an apoptosis regulator, which promotes its release, the intrinsic pathway is linked to increased mitochondrial membrane permeability and cytochrome c release. After then, activation of caspase triggers activation of caspase 9, which cleaves procaspase-3 downstream, activating caspase 3 and inducing cytotoxicity. The extrinsic pathway is triggered by the overexpression of FasL, which attaches to the Fas receptor, a surface-located protein of the cytotoxicity-inducing receptors family. Poly ADP-ribose polymerase (PARP), lamin, caspase-activated DNase inhibitor (iCAD), and the protein 8-related to XK (XKr8) are among the substrates of caspases 3 and 7. By affecting several apoptotic processes, including as nuclear condensation, DNA fragmentation, membrane blisters, and phosphatidylserine exposure, caspases cleaves these substrates.[87]

Numerous species of scorpions have venoms that affect the apoptotic intrinsic process. Bax, caspase 3, and caspase 9 were overexpressed in *R. junceus. A. amoreuxi* increased the synthesis of caspase 3 and adversely regulated Bcl-2. *B. martensii Karsch* downregulated Bcl-2 and expressed caspase 3. BmKn-2 inhibited Bcl-2 and activated caspases 3 and 9. The activities of caspase 3 and Bcl-2 were downregulated in fraction III. *Bengalin* triggered caspase 3, rAGAP expressed Bax, and LMWSVP both expressed and inhibited Bcl-2. FasL expression rose in response to neopladine 1 and 2, but not in response to rNeo2a.[88]

The capacity of crude venom, toxins, or separated components to stop the cell cycle at specific phases (G0/G1, G2/M, and G1/S) is another feature. Its progression throughout the G2/M transition is primarily regulated by the cyclin-dependent kinase 1 (CDK1)/Cyclin B complex. The cyclin-dependent kinase inhibitor p21 is a crucial regulator that inhibits CDK1 activity and stops the G2/M phase, which explains why the formation of cancer cells is prevented. *Heterometrus tangi* and *B. martensii Karsh's* venoms were in charge of p21. It was also discovered that the presence of *R. junceus* venom increased the expression of tumor protein 53 (p53). Protein 53 is one protein that has the potential to cause cell cycle arrest (mostly via p21 activation). During the G0/G1 transition, the CDK2/Cyclin A complex is necessary for cell cycle progression. Cell cycle stages could be stopped by the p27 protein.[89]

In a previous study evaluating the capacity of the crude venom and isolating toxins to stop the cell cycle the result was that *A. crassicauda* triggered cell cycle arrest in the S phase. *B. martensii* halted the cell cycle in the G1 and S phases, while *H. bengalensis* did so in the sub-G1 phase. While rAGAP and component III of scorpion venom caused cellular arrest in the G1 phase, *bengalin* and Cn2 halted the cell cycle in the sub-G1 and G0/G1 stages, respectively.[73] Cytotoxicity is partially attributed to voltage-activated ion channels. Chloride (Cl) channels in glioma cell membranes are upregulated in response to DNA damage, which improves cell survival and Cl transport. Aquaporins in migrating cells facilitate osmotic water flow through ion flux from these channels. Human glioma cells' leading edge NKCC1 encourages Cl accumulation, which produces an electrochemical driving force. By osmotically controlling cytoplasmic water, this Cl flux alters morphology and promotes metastasis. rBmkCTRat glioma C6 cells bind to a toxin from *B. martensii Karsch*, which blocks Cl channels and stimulates cell proliferation. Additionally, this

toxin binds to MMP-2 in the extracellular matrix and inhibits it a process that is aided in tumor cell movement and invasion by proteins creating a complex.[90] On the other hand, the rat glioma C6 cell line was previously used to investigate the use of a graphene oxide fraction generated from *L. quinquestriatus* venom that was conjugated with chlorotoxin.[91]

It is believed that potassium channels are crucial for cancer cells. The characteristics of malignant transformation, including rapid growth, loss of inhibition by cell contact, and sensitivity to anti-growth signals, have been linked to the overexpression of these channels. We have investigated the overexpression of human ether-a-go-go-related gene (hERG) channels in hematopoietic cancers. It was discovered that BmKKx2 from *B. martensii Karsch* could block hERG channels and start the cell cycle in the G1 phase. Additionally, it was shown that BmKKx2 therapy led to cell differentiation, which was commonly followed by apoptosis. Inducers of apoptosis are more likely to cause leukemic cells to undergo differentiation.[92] On the other hand, several toxins isolated from scorpion venom encourage the growth of cancer cells. The isolation of AcrAP1 from *A. crassicauda* improves the growth rate of PC-3 and NCI-H460 cancer cell lines by 35%-45%. Similar to this, pET-28a-Sj7170 which was obtained from *Scorpiops jendeki*, boosted U-87 (human glioblastoma) cell line proliferation by 75% when compared to the control group. It caused the synthesis of cyclin D1 and increased the number of cells in the S phase.[93]

THE ROLE OF SCORPION VENOM TOXINS IN INDUCING CYTOTOXIC EFFECTS

In a previous study TsAP-S1 fraction demonstrated significant cell death against NCI-H157 (human squamous cell carcinoma), NCI-H838 (human lung adenocarcinoma), U-251 (human glioblastoma), PC-3, and MCF-7 cell line. TsAP-S2 also demonstrated high cell death rates against NCI-H838, U-251, NCI-H157, PC-3, and MCF-7 cell lines; TsAP-1 was tested on HCI-H838 and NCI-H157 cell lines, revealing moderate cell mortality; TsAP-2 was evaluated against NCI-H838, U-251, PC-3, MCF-7, and NCI-H157 cell lines, revealing substantial cell death.[94] When *B. martensii Karsch* venom was tested against an oral cancer cell line that had a 95% death rate in less than 24 hours, its anticancer properties were found. Scorpion venom has the ability to interacts with and targets cancer cells. It was discovered that a venom fraction had tumoricidal effects against the human hepatocellular carcinoma HepG2 cell line, with an IC50 value of 200 mg and a death rate of 91.2%. *A. mauritanicus* venom produced a toxin called mauriporin, which showed tumoricidal effects against PC-3 (human prostate adenocarcinoma) and LNCaP and DU 145 cell lines (prostate cancer) with notable death rates.[79]

In a previous study using SW-480 cells (colorectal adenocarcinoma), rAGAP from *B. martensii Karsch* venom showed strong anticancer activity against it. Rat glioma C6 cells treated with LiCl and plasmid pEGFP-N1-BmK, which carried the BmK CT gene, showed significant cytotoxicity within a 24 hour. The same venom's purified SVIII toxin shown efficacy against both Jurkat cells (acute T cell leukemia) and THP-1 (acute monocytic leukemia). In C6 cells, the I-BmK CT percentage likewise shown significant mortality. *H. bengalensis* venom's bangalin toxin showed anticancer action against K-562 (chronic myeloid leukemia) and U-937 (histiocytic lymphoma) cells, resulting in a notable 24-hour cell death. The venom of *B. martensii Karsch* contained BmKn2, which demonstrated efficacy against colonic cancer SW620 and HSC-4. When applied to MCF-7 cells, VmCT1 from *Vaejovis mexicanus smithi* venom proved tumoricidal.[95]

CONCLUSION

A wide range of bioactive chemicals with a strong affinity for various targets in cells and tissues make up animal venoms. Isolated peptides and scorpion venom possess strong antitumor effects against various cell lines which show promising use as anticancer agent. Through inducing apoptosis, they have proven to have exceptional cytotoxic efficacy against a variety of cancer cell types. Moreover, venoms have a special potential to obstruct ion channels in cancer cells, which suppresses particular characteristics. Furthermore, it is known that venoms and peptides can induce cell cycle arrest at the beginning of the G1, G2, and S phases, which stops cancer cells from multiplying out of control. The anticancer qualities of scorpion venoms against a variety of cancer cell lines utilizing suitable model organisms require more prior in vivo research before using it as a treatment. which can surely lay a strong foundation and open a new avenue for the scientific communities towards cancer treatment in the future. Primarily, anticancer medications linked to scorpion toxins can only be developed and released into the market if they show no harm to normal human cells during clinical testing stages as chlorotoxin that shows few side effects on normal cells, so it is being used as a treatment with many clinical trials have been on it.

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