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Anti-tumoral effect of scorpion peptides: Emerging new cellular targets and signaling pathways

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ABSTRACT

Scorpion toxins have been the subject of many studies exploring their pharmacological potential. The high affinity and the overall selectivity to various types of ionic channels endowed scorpion toxins with a potential therapeutic effect against many channelopathies. These are diseases in which ionic channels play an important role in their development. Cancer is considered as a channelopathy since overexpression of some ionic channels was highlighted in many tumor cells and was linked to the pathology progression.

Interestingly, an increasing number of studies have shown that scorpion venoms and toxins can decrease cancer growth *in vitro* and *in vivo*. Furthermore through their ability to penetrate the cell plasma membrane, certain scorpion toxins are able to enhance the efficiency of some clinical chemotherapies. These observations back-up the applicability of scorpion toxins as potential cancer therapeutics.

In this review, we focused on the anti-cancer activity of scorpion toxins and their effect on the multiple hallmarks of cancer. We also shed light on effectors and receptors involved in signaling pathways in response to scorpion toxins effect. Until now, the anticancer mechanisms described for scorpion peptides consist on targeting ion channels to (i) inhibit cell proliferation and metastasis; and (ii) induce cell cycle arrest and/or apoptosis through membrane depolarization leading to hemostasis deregulation and caspase activation. Putative targets such as metalloproteinases, integrins and/or growth factor receptors, beside ion channels, have been unveiled to be affected by scorpion peptides.

1. Introduction

Animal's venoms peptides are known to exhibit therapeutic role by diversified mechanisms of action which makes them unexampled agents in a comparison with existing commercial drugs. They show selectivity in targeting cancer cells without damaging untransformed cells. Thus many reviews highlight the identified venom peptides and proteins from different venomous animals like snakes, scorpions, spiders, bees, wasps, snails, toads, frogs and sea anemones and their anticancer activities as well as the identification of involved signaling pathways are well documented [1–5]. Each of these molecules has a specific activity making them important to elucidate parts of carcinogenesis mechanisms. In this review we focus on scorpion peptides and their targets in cancer cells.

Scorpion toxins are generally positively charged (basic) molecules sharing structural homologies and physico-chemical properties. Despite this homology scorpion toxins cover a wide spectrum of pharmacological effects allowing them to be an effective defense tools with a synergistic effect against the scorpion's prey. Structure and function of scorpion toxins were studied for many years in order to elucidate the mechanism of their toxic effect and to conceive serotherapy against scorpion envenomation.

The specific interaction with ion channels was shown to be the major mechanism behind the pharmacological effects of scorpion toxins. Because of their strong affinities towards ion channels, scorpion toxins were used as probes to determine the density of these specific receptors in various tissues [6]. They are also used as specific and selective ligands to study the location, the structure and/or the functions of ionic channels [7,8]. Since ionic channels are involved in many diseases, scorpion toxins are actually investigated as a source of potential therapeutic agents [9–11]. Thanks to their small size, the majority of scorpion toxins have the advantage to be chemically synthesizable in an active form, promoting their use as valuable tools and lead compounds for new drug development [12].

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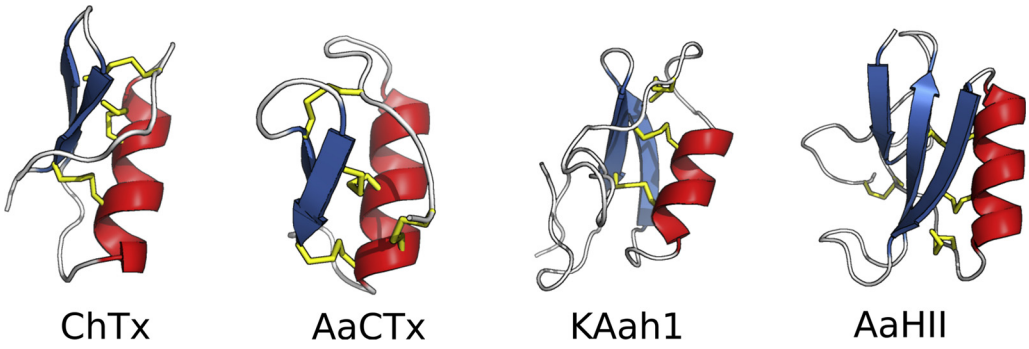

Fig. 1. 3D structures of different types of scorpion toxins.
ChTx : Charybdotoxine from *Leiurus quinquestriatus quinquestriatus* scorpion venom active on K^+ channels [16]; AaCTx : from *Androctonus australis* scorpion venom active on Cl^-channels [11]; KAaH1 from *Androctonus australis Hector* scorpion venom active on K^+ and Na^+ channels [20]; AaH II from *Androctonus australis Hector* scorpion venom active on Na^+ channels [16].

2. Scorpion toxins classification

Scorpion toxins were first broadly classified into two families: "short" toxins, constituted of 30–40 amino acids (AA) and "long" toxins of 60–70 AA. Although these toxins have different sequences [13], most of them adopt the common Csαß (Cysteine-stabilized α/ß motif) fold formed by a helix and 2 or 3 ß strands and stabilized by three of four disulfide bridges [14,15] (Fig. 1).

Due to their important affinity for ionic channels, the diversity of scorpion toxins is closely related to their pharmacological targets. Thus, scorpion toxins are also classified in several categories according to their targeted channel. Short toxins generally bind to potassium calcium or chloride channels, whereas most long toxins are active on voltage gated sodium channels. The topology of a site, structured by a characteristic peptide sequence, is one of essential conditions for the existence of a specific biological activity. The information concerning relationships between the architecture of the active site and the specific activity led to the notion of "structure-function relationship".

2.1. Sodium channel scorpion toxins

Sodium channel scorpion toxins (NaScTxs) are mostly responsible for the neurotoxic effect of scorpion venoms. These toxins are classified according to their sensitivity to the voltage changing upon the binding to the target. Therefore, we distinguish the α-type and ß-type toxins for sensitive and insensitive to the voltage change respectively [17]. Both α- and β-NaScTxs are appropriately described as Nav channels activators albeit with a distinct mechanism of action.

NaScTxs are also classified according to their targeted organism: toxins specific for mammals, insects, or those simultaneously active on several different organisms [18]. This classification is also related to sequence similarities which are closely related to their activities. We distinguish 10 groups of long chain scorpion toxins. The first 4 groups contain the α-type toxins, the groups 5 and 6 contain β-type toxins, and the groups 7, 8 and 9 correspond to anti-insect toxins. The last identified group (group 10) contains Birtoxin-like toxins (Fig. 2). These are 58 amino acids peptides with only three disulfide bridges [19–21]. This group contains peptides with similar sequences but different activities: some of them are active on Na^+ channels [19,21], while the others are active on K^+ channels and very weakly active on Nav channels [20]. It could thus represent an intermediate group between toxins active on sodium channels and those active on potassium channels [22].

2.2. Potassium channel scorpion toxins

Potassium channel toxins (KTxs) are poorly represented in scorpion venoms. They are short peptides (compared to long toxins active on sodium channels) constituted of 30–40 amino acids. Some of them have a very high affinity (in the order of the pM or nM) for one or more subtypes of potassium channels. These peptides are cross-linked by three or four disulfide bridges that allow the molecule to have stable three-dimensional conformation [15].

The classification of KTxs, according to their sequences was first established by Miller [23]. This classification is based on the alignment of the cysteine and others highly conserved residues. The number of scorpion venom peptides reported to block or modify the permeability of potassium channels in excitable and non-excitable cells strongly increased last years. Today, all KTx are proposed to be grouped into 6 families based on homology, 3D folding pattern, and activity. CSα/β toxins are divided into three families: α-KTxs (20–40 residues), β-KTxs (45–75 residues) and γ-KTxs (affecting a particular subset of Kv, so-called ERG channels). CSα/α toxins are placed in the φ-KTxs family. Kunitz toxins are named *δ*-KTxs and recently ICK toxins have been proposed to constitute the κ-KTxs family [24]. The α-KTxs family is considered as the most important and counts about 200 peptides [24]. They are divided in over 31 subfamilies (α-Ktx1 to α-Ktx31; the subfamily α-Ktx25 is empty) [25,26] (Fig. 3). These toxins present a consensus sequence allowing to establish a structural common motif. Indeed, the positioning of 6 cysteine residues is identical in all the toxins (Fig. 3), which could be on the basis of the formation of the architectural α/ß motif [27]. This architecture imposes constraints, and a consensus proposed by Bontems et al. [28], setting the position of 7 residues:

-C-[…]-C-X-X-X-C-[…]-G-X-C-[…]-C-X-C

2.3. Chloride channel scorpion toxins

The first identified chloride channel blocker toxin is Chlorotoxin (ClTx), isolated from the *Leirus quiquestriatus* scorpion venom [29]. It is a short peptide of 36 amino acids residues [30] cross-linked by four disulfide bridges. ClTx was initially used as a pharmacological tool to characterize chloride channels. Other chlorotoxin-like peptides were identified such as BmK-CT (or Bm-12 (Fig. 4)), purified from *Buthus martenzii Karsch* scorpion venom [26] [31]. The mRNA corresponding to the precursor of a second chlorotoxin-like peptide was found in the same venom called rBmK-CTa (or Bm-12b (Fig. 4)) [32]. These peptides showed 66% sequence identity with ClTx. GaTx1 a peptide of 3.7-kDa, from the same scorpion venom, is also active on chloride channel [33]. It was reported that ClTx and BmK-CT block the small conductance chloride channels [29,34], whereas GaTx1 is validated as a highly-specific blocker for the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel, despite its close sequence similarity to ClTx (75%) [33]. Other chlorotoxin-like peptides are listed in Fig. 4 but their effects on chloride channels have not been demonstrated.

2.4. Calcium channel scorpion toxins

The first identified scorpion toxin, active on calcium channel is Imperatoxin A (IpTx A), isolated from the venom of *Pandinus imperator* [35]. Several IpTxA-like peptides were then identified from other scorpion venoms, including Maurocalcine (MCa) [36], Hemicalcin (HCa) [37] and Hadrucalcin (HdCa) [38]. They consist of 33–35 amino acids, including 6 cysteine residues. Two other toxins, BjTx-1 and BjTx-2, of 28 amino acids were isolated from the venom of the scorpion

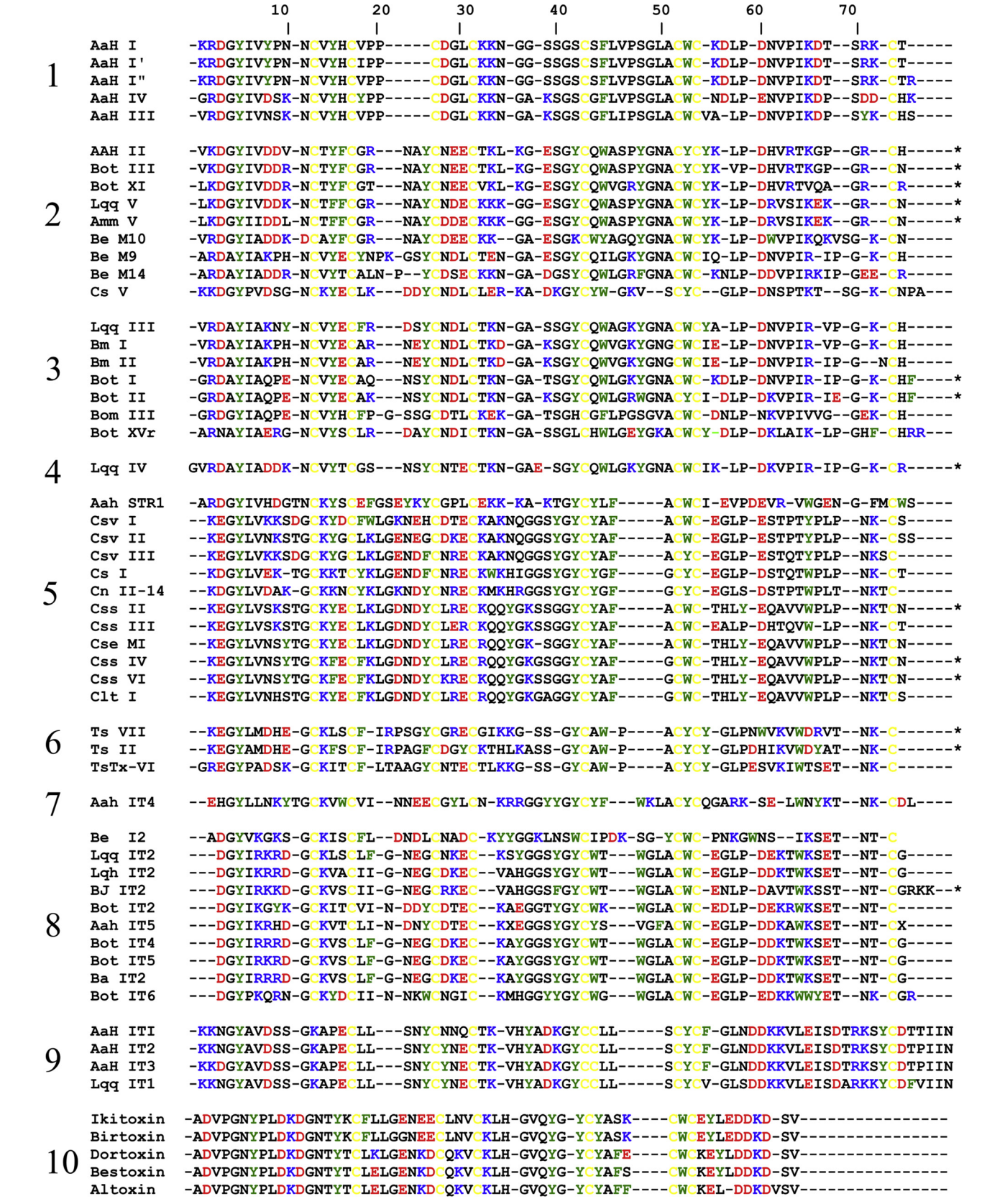

Fig. 2. Amino acid sequences of some scorpion toxins active on sodium channel.
Sequences were aligned taking as reference the Cys residues (yellow colored). Gaps were introduced in order to maximize similarities. The asterisk indicates C-terminal amidation. Amino acids are colored according to their physico-chemical characteristics: red (acid), blue (basic), green (aromatic). The group number (see text) is indicated at the left side of the figure.

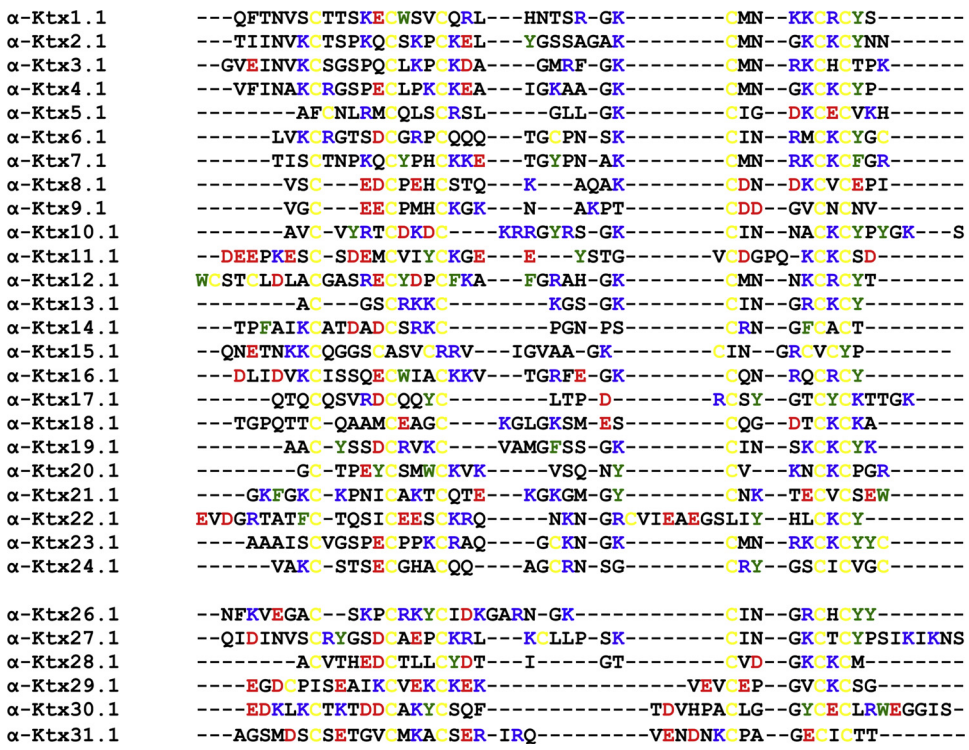

Fig. 3. Amino acid sequences of the first member of each family of α-Ktx toxins.
Sequences were aligned taking as reference the Cys residues (yellow colored). Gaps were introduced in order to maximize similarities. Amino acids are colored according to their physico-chemical characteristics: red (acid), blue (basic), green (aromatic).

Fig. 4. Amino acid sequences of scorpion toxins active on chloride channels.
Sequences were aligned taking as reference the Cys residues (yellow colored). Gaps were introduced in order to maximize similarities. Amino acids are colored according to their physico-chemical characteristics: red (acid), blue (basic), green (aromatic).

Buthus Judaicus [39] (Fig. 5A).

All these peptides stimulate the interaction of [^{3}H]-ryanodine with RyR1 (Ryanodine receptor type 1) and result in significant release of Ca^{2+} in sarcoplasmic reticulum (SR). On the other hand, these toxins stabilize the state of conductance extension in the RyRs of the skeletal muscle reconstituted in the bilipid layers. Other peptides, such as Opicalcin 1 and 2 have been deduced from a nucleotide sequences of *Opistophthalmus carinatus* scorpion venom cDNA libraries [40] (Fig. 5A). Those calcins have not yet been purified nor tested for their biological activity.

The 3D structures of MCa and IpTxA were determined by NMR [41,42]. These toxins are folded according to an ICK (Inhibitory cystine Knot) pattern different from the conventional CS/αβ fold characteristic of scorpion toxins active on Na^+, K^+ or Cl^- channels (Fig. 5B). The term "Knot cystine" or cystine knot consists of a ring formed by two disulfide bridges having their peptide chains crossed by a third disulfide bridge. This pattern is found in several structures of cysteine-rich peptides, and marine cone toxins [43–47]. All these molecules share the same disulfide bridge pairing pattern: C1-C4/C2-C5/C3-C6 (cysteines 1 to 6 are numbered in ascending order from N-terminal to C-terminal) with C3-C6 being the crossing ring bridge. Matching the disulfide bridges is necessary but not sufficient to observe the formation of cystine nodes. The predominant secondary structural element in these toxins is a two or three stranded sheet linked with the cystine node (Fig. 5B).

Another family of calcium channel toxins was identified from the venom of the *Parabuthus* scorpion species. These are called Kurtoxin and Kurtoxin-like peptides composed of 63 amino acids and shown to bind with high affinity to T-type voltage-gated calcium channels (Cav3.x) [48]. These toxins interact also with, L-, N-, and P-type Cav

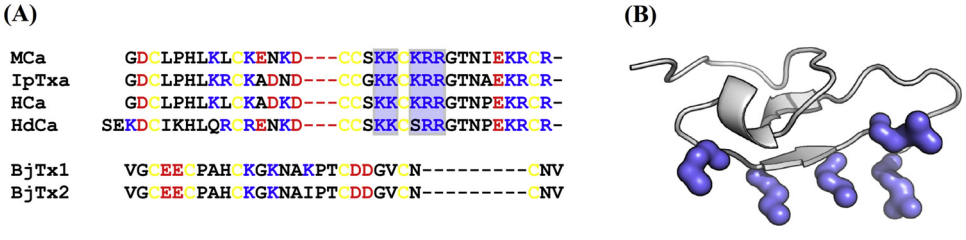

Fig. 5. Structures of scorpion toxins active on calcium channels.
(A) Primary structures : Sequences were aligned taking as reference the Cys residues (yellow colored). Gaps were introduced in order to maximize similarities. Amino acids are colored according to their physico-chemical characteristics: red (acid), blue (basic), green (aromatic). (B) 3D structure of Maurocalcine (PDB code 1C6W) that interacts with the RyR channel through a number of basic residues (residues in blue) clustered in one surface.

channels [49], as well as Nav1.6 channel [50] in central and peripheral neurons.

2.5. Other groups of scorpion venom molecules

Scorpion venom contains other peptides/proteins classes beside neurotoxins. Indeed non-disulfide-bridged peptides (NDBPs) [51] were also identified in scorpion venoms. It also contains enzymes like phospholipases A2 [52–54], acetylcholinesterases, hyaluronidases [55], alkaline phosphatases and proteolytic enzymes with strong cytotoxic and gelatinolytic activities like L-amino acid oxidases and proteases such as serine proteases [56] or metalloproteinases [57–59]. All these proteins have different pharmacological effects such as antibacterial [60], antifungal, antimalarial, antiviral and anticancer activities [61].

3. Scorpion toxins affecting hallmarks of cancer and their targets

Scorpions and their venoms have been used as traditional and folk therapy in various pathophysiological conditions in India, China, Africa and Cuba. The anticancer potential is a recently described biological property of scorpion venoms and toxins. Several studies have shown that scorpion venoms and toxins could impair tumor growth, induce apoptosis and inhibit cancer metastasis and angiogenesis *in vitro* and *in vivo* [11,62,63]. Interestingly, the mechanism of action of certain toxins is similar to that of some agents currently used in chemotherapy. Despite the extensive work on characterizing the molecular targets of hundreds of scorpion peptides, the effect on different hallmarks of cancer was evaluated only for few of them. Cell targets of the peptides were relatively well studied and we currently dispose of several interaction models that could explain their mode of action. Nevertheless, fewer studies have been proposed to detail the causality relationship of targeting a specific channel and the effect on a cancer cell hallmark. Such relationship could be abstracted according to the molecular target and cell cancer hallmark.

It is worth noting, at this level, that the models we are exposing are established only for scorpion peptides for which the physical target and/or the in vitro anti-cancer assessment are well documented.

3.1. Targets of sodium channel scorpion toxins in cancer cells

Emerging preclinical data suggest that pharmacologically targeting voltage-gated Na^+ channels (VGSCs) may reduce local invasion and metastasis in mouse models [64,65]. The potential utility of VGSC-inhibiting agents as anti-metastatic therapies has not surfaced in the clinic. However, the preclinical data raise the intriguing possibility that cancer patients taking VGSC-inhibiting medication for other pre-existing indications, e.g., epilepsy, may have improved cancer-specific outcome compared with control groups not taking such treatments [66,67]. Indeed, in some tumor cells, the functional activity of Nav channels appeared to be involved in regulating the proliferative, migratory, and invasive properties of cells [63–67] [68–72] and proposed them as therapeutic targets [73]. Furthermore, it has been shown that metastatic cells expressed high levels of VGSCs in prostate [74], breast [75,76] and melanoma [68,77–79]. The abnormal expression of Nav channel proteins in carcinoma cells were thus associated with aggressive features; while the cellular mechanisms proposed to be involved remained elusive.

Human Nav channels are divided into nine subtypes (Nav1.1–1.9), each one with diverse roles and locations [80,81]. Expression levels of Nav1.6 and Nav1.7 were reported to be 6–27 times higher in PC3 and LNCaP cells, compared to normal and benign hyperplastic prostatic tissues [82]. Nav1.1,Nav1.2 and Nav1.9 isoforms were highly expressed in hormono-independent cancer cell lines DU145, PC3 and PC3 M in comparison with hormono-dependent cells LNCaP, C4-2, C4-2B and CWR22rv- [83]. However, the expression of Nav1.5, Nav1.6 and Nav1.8 was noticed in all prostate cancer cells. Furthermore, Nav1.8 is strongly expressed in advanced pathological stages of prostate cancer [83]. Therefore, Nav1.8 was suggested as potential biomarker to differentiate between early and advanced stages of disease [83], but its functional mechanism of in prostate cancer is not well defined.

Sodium currents can be modulated by many toxins that interact with Nav channel proteins at different binding sites. According to the resultant effects on macroscopic Nav channels currents, these toxins can be classified as channel inhibitors or activators. The well-known inhibitor is tetrodotoxin (TTX), a potent marine neurotoxin [84–87]. While all Nav channel isoforms can be blocked by TTX, two families of channels have been identified based on their sensitivity to TTX. The "TTX-sensitive channels" includes Nav1.1–1.4, Nav1.6 and Nav1.7 that can be blocked by nanomolar concentrations of TTX. The so-called "TTX-resistant channels" Nav1.5, Nav1.8, and Nav1.9 required micromolar concentrations to be blocked. Nav1.8 channel is the most insensitive as it has a TTX IC_{50} of 50 μM [88].Blocking VGSC activity with TTX has been shown consistently to suppress a range of metastatic cell behaviors, including motility, and cell invasion of different highly metastatic prostate cancer cell lines [89–92]. However TTX did not induce changes in the proliferation of these cell lines even at higher doses [91–93], suggesting that Na^+ channel activity could contribute significantly to the metastatic potential of the tested cells [94].

Interestingly proliferation of different tumor cells was inhibited by NaScTxs. Indeed the peptide Cn2 from the *Centruroïdes noxius* scorpion venom was shown to decrease the cell proliferation by inducing cell cycle arrest and early apoptosis of mouse neuroblastoma F11 cells [95]. This peptide was shown to induce both the left shift voltage-dependent activation and a transient resurgent current only in human Nav1.6 channels in cerebellar Purkinje neurons [96].

Recombinant rBmKAGAP and BmK AGAP-SYPU2 toxins from the venom of *Buthus martensii karsch* scorpion, showed antitumoral activity in mouse S-180 fibrosarcoma models *in vivo* [97,98]. rBmK AGAP inhibited the proliferation of SW480 human colon cancer cells by inducing cell cycle arrest in the G1 phase and cell apoptosis through upregulating the expression of p27, Baxand PTEN along with decreasing Bcl-2 level and PI3K and Akt phosphorylations [99]. The recombinant toxin inhibited also the proliferation of SHG-44 cells by arresting the cell cycle. On another hand, rBmKAGAP seems to affect also the metastatic cell behaviors. Indeed it inhibited the migration of SHG-44 cells by inducing inactivation of AKT, p-38, Erk1/2 and c-Jun kinases and decreasing the VEGF and MMP-9 protein levels [100,101]. Furthermore, recent study by Kampo et al. [102], showed that rBmK AGAP inhibited stemness, epithelial-mesenchymal transition (EMT), migration, and invasion of mammary tumor cells MCF-7 and MDA-MB-231 *in vitro* and *in vivo* by down-regulating the inflammatory mediator PTX3, over expressed in breast cancer, through NF-κB and Wnt/β-catenin signaling pathways [102]. Electrophysiological analysis revealed that this peptide potently inhibited Nav1.8 currents and negatively shifted the voltage dependent threshold of the activation and inactivation. It also reduced the NaV1.9 currents, but had no significant effect on activation and inactivation kinetics [103]. On another hand, rBmKAGAP was shown to affect Na^+ current by inhibiting the Nav1.5 mRNA expression, resulting in down-regulation of the channel activation. Expression of this channel as well as Nav1.7 isoform was shown to be particularly related to cancer cell migration [104–107].

Thus, it appears that a complex regulation signaling mechanism involving different isoforms of VGSCs, is implicated in the effect of NaScTxs. This effect could be related to the activation/inhibition and/or expression of these VGSC isoforms.

On the other hand, Fraser et al. (2000) showed that TTX (6 μM) blocked completely the voltage-gated Na^+ channel selectively expressed in the strongly metastatic MAT-LyLu prostatic cancer cell line, but had no effect on the proliferation of neither these cells nor on the weakly metastatic AT-2 rat prostatic cancer cell line, whereas veratridine (10–50 μM) the presumed Na^+ channel opener, reduced significantly, the proliferation of both cell lines by up to 30% [72].

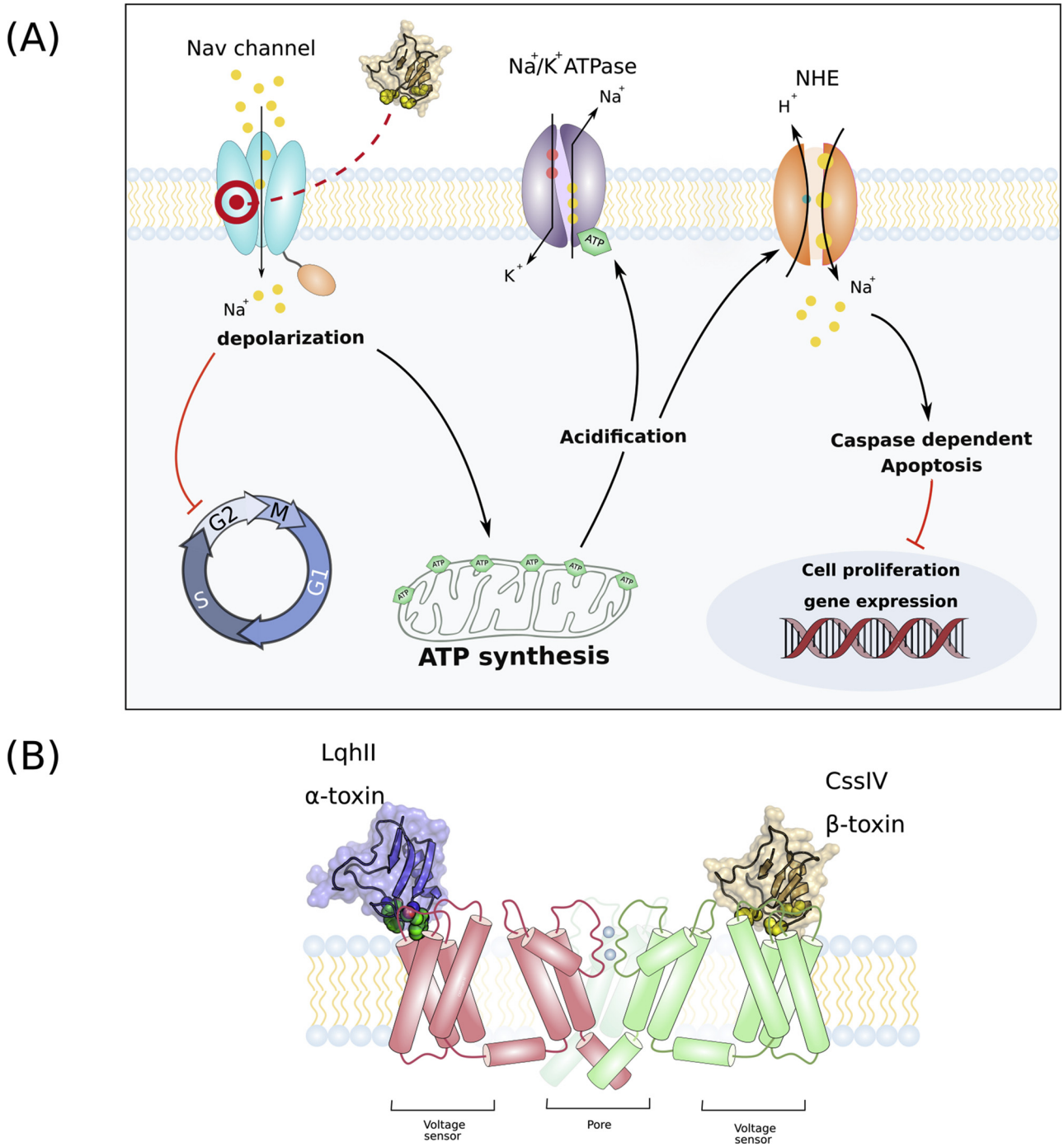

Fig. 6. Modulation of cellular targets involved in cancer cell proliferation and apoptosis mechanisms by NaSCTx.
(A) Signaling pathways involved in the mechanism of action of the scorpion peptide inhibiting VGSCs. Cell depolarization is still maintained after the scorpion peptide interact with the sodium channel. However, the peptide perturbs the equilibrium of the activation of the channel which in turn disorder all the ion concentrations in the cytoplasm. Two signaling pathways are illustrated to explain the anti-apoptosis and the anti-proliferation effects. Anti-proliferation activity in particular can result from the anti-apoptosis triggering and the cell cycle progression arrest. (B) Mechanism of VGSC modulation by an α-toxin (LqhII) and a β-toxin (CssIV) by interacting with voltage sensor domain.

In regard to several studies [75,96,99,102,103], highlighting different effects of NaScTxs, TTX and veratridine, on hallmarks of cancer cells, we can hypothesize that proliferation of tumor cells and/or cell death are closely related to opening of specific VGSCs isoforms, such as Nav1.6 or Nav1.8, and consequently Na^+ influx. Two scenarios could be proposed based on the effect of scorpion toxins on the cell signaling pathway components. The first one is that once the scorpion peptide interacts with the Na^+ channel, the depolarization is maintained following the continuous influx of Na^+ ions. This event decrease the transient electrical charge (depolarization) and prevent return to repolarization in the G0/G1 phase [108] (Fig. 6A), thus altering the key control points of the cell cycle [109,110] and consequently the cell proliferation.

Another scenario, suggests that the elevated intracellular concentrations of Na^+ ($[Na^+]_i$) play a critical role in controlling cell size and apoptotic cell death [111]. There is strong evidence that the effect of scorpion peptide on VGSC might implicate a cell cytoplasm acidification mechanism similar to that of Veratridine [112]. In fact, it is

known that VGSCs regulate intracellular H^+ concentrations [113,114]. While still poorly explored, the mechanism is, however, thought to be tightly linked to glycolysis [115]. In particular Veratridine activity was shown to correlate with increased respiration the lactate production in the cell [112]. When trying to re-establish the resting potential, the cell involves the Na^+/K^+ ATPase pump to regain the physiological concentrations of Na^+ and K^+ in the cytoplasm. Such membrane proteins require the supplementation with ATP provided by the aerobic and anaerobic glycolysis pathways which in return induces the elevation of intracellular H^+ concentration.

Therefore, the activation of the channel leads to cell interior acidification which in turn activates the Na^+/H^+ exchangers [116]. The concentration of Na^+ will, therefore, increase in the cytoplasm and an imbalance of K^+, Ca^{2+}, and Na^+ ion concentrations will be created. The effect of this deregulation could activate the caspase pathway leading to cell apoptosis [116,117]. However, it is still unclear whether the route of entry of Na^+ influences is directly related to caspase activation [118–121].

It is consistent to consider the effect of scorpion peptides as a disturbing element of sequential cellular events in which the cancer cell requires signaling control in space and time to regulate hemostasis and ensure its survival. Nevertheless, much more efforts are needed by studying as many NaScTxs as possible to elucidate and clarify the mechanism of these peptides. In fact, until now only few NaScTxs have been tested on cancer cells. As far as we know, Cn2, rBmKAGAP and BmK AGAP-SYPU2are the only NaScTxs tested on tumor cells, while many other NaScTxs might be candidates for anticancer therapy. The Table 1 summarizes the NaScTxs that could be potentially active on tumor cells through modulating sodium channel subtypes expressed in different cancer cell lines [68]. They include either α-NaScTxs or β-NaScTxs, which interact with voltage-gated sodium channels (Navs) at two pharmacologically distinct sites localized on extracellular face of the channel, altering their voltage dependent gating state. α-NaScTxs bind at receptor site-3 and inhibit channel inactivation, whereas β-NaScTxs bind at receptor site-4 and shift the voltage-dependent activation toward more hyperpolarizing potentials (Fig. 6B).

A functional surface called “NC-domain” was identified in α-NaScTxs (revised in [128,132,133]. This domain comprises the five-residue turn (residues 8–12) that interlaces with the C-terminal segment and a core-domain formed by several positively-charged and hydrophobic amino acids. The NC- and core-domains are interconnected by the linker-domain residues 8–18. The NC-domain varies in amino acid composition and spatial arrangement which likely dictates toxin selectivity [128,132–135].

For β-NaScTxs, four common functional sections were identified: 1) a central “pharmacopore region” involved in the receptor binding site, consisting of a negatively charged residue, located in the α-helix which is flanked by diverse solvent-exposed hydrophobic residues; 2) a “solvent-exposed aromatic cluster” that is critical for activity and is generally located in the β2 and β3 strands; 3) some residues localized in the center of the N-groove region, which are involved in voltage sensor trapping; and 4) C-terminal residues that increase the affinity to the receptor binding site (reviewed in [136]). For identification of functional regions of the β-NaScTxs subclasses, four motifs have been proposed [136,137]. Few structure-function relationship studies were performed for NaScTxs against Nav1 channel subtypes [138]. Recently, Clairfeuille et al, [139] submitted the first experimentally solved complex of an α-toxin, AaH2, and a chimera protein Nav1.7 voltage sensor domain (Nav1.7-NavPas), by cryo electron microscopy. The structure confirms previous findings about AaH2 amino acids implicated in its interaction with Nav channels and also highlights the binding of the toxin to two different voltage sensor sites. It is not known if this property could be generalized to other α-toxins, but this could be a promising result to motivate other works to be carried on scorpion peptides.

3.2. Targets of potassium channel scorpion toxins in cancer cells

Many K^+ channel scorpion toxins were shown to inhibit different cancer hallmarks. More interestingly, some of them allowed elucidating the functional role of K^+ channels in the neoplastic progression steps of different cancer cell lines [140].

KAaH1 and KAaH2 are two homologous peptides from *Androctonus australis* scorpion venom [20] that block differently the voltage gated potassium channel (Kv) subtypes Kv1.1 and Kv1.3. These peptides also differently inhibit the progression steps of three aggressive cancer cell lines. KAaH1, active on Kv1.1 and Kv1.3 channels, inhibits the adhesion of U87 glioblastoma cells and the migration of U87, MDA-MB-231 (breast cancer) and LS174 (colon adeno carcinoma) cells. Whereas KAaH2, which is slightly active only on Kv1.1 channel, inhibits the U87 cell proliferation via the EGFR signaling pathway [141]. The specific KAaH2 anti-tumor activity on U87 cells, suggests that this peptide might act on cell proliferation by interacting with the EGFR directly, or via a K^+ channel other than Kv1.1 and Kv1.3 channels [140]. Kv11.1 channel could be a potential candidate, since EGFR has been reported to form a multimeric complex with this channel [141]. The interaction between Kv11.1 and EGFR is based on phosphorylation/dephosphorylation reactions at the Tyr475 located on the intracellular S2-S3 loop of the channel [142].

The effect of KAaH1 on cancer cells adhesion and migration could

Table 1
Scorpion toxins affecting voltage-gated Na^+ channels shown to be expressed in different cancer cell lines: Prostate (PC3, 22Rv1 ; DU-145 and/or LnCap), Breast Cancer (MDA-MB- 231 and/or MCF-7), Lung (H69, H209, H510, H460, Calu-1, H23 and/or A549), Leukemia (Jurkat) Mesothelioma (Primary-Cultured human, malignant pleural mesothelioma cells), Cervix (Primary cultured human cervical carcinoma cells), Ovary (Anglne, Caov-3, and/or SKOV-3); Colon (HT-29,SW620 and/or SW480).

Toxin	VGSC isoforms targetted	Cancers expressing VGSC isoforms [71]
Bot IX; BmKαIV	Nav1.2 [122,123]	Prostate, lung, Mesothelioma, Ovary
Ts4; Kurtoxin	Nav1.6 [124,50]	Prostate, Breast, Lung, Leukemia, Mesothelioma, Ovary, Cervix
BmK AS	Nav1.3 [125]	Prostate, Lung, Mesothelioma, Ovary, Cervix
BmKM1; AmmVIII	Nav1.7 [126,127]	Prostate, Breast, Lung, Leukemia, Mesothelioma, Cervix
LqhαIT	Nav1.2 [128] Nav1.4 [129]	Prostate, Lung, Mesothelioma, Ovary, Cervix
CssIV	Nav1.2, Nav1.5 [129]	Prostate, Breast, Lung, Leukemia, Mesothelioma, Ovary, Colon
Lqh-2; Lqh-3	Nav1.2; Nav1.7 [129]	Prostate, Breast, Lung, Leukemia Mesothelioma Cervix, Ovary
CssII	Nav1.5; Nav1.6 [130]	Prostate, Breast, Lung, Leukemia Mesothelioma Cervix, Ovary, Colon
Lqh-3; Lqh-2	Nav1.2; Nav1.4; Nav1.7 [129]	Prostate, Breast, Lung, Leukemia, Mesothelioma, Ovary, Colon, Cervix
Cn8; CII1; CII2	Nav1.1 to Nav1.5 [130]	Prostate, Breast, Lung, Leukemia, Mesothelioma, Ovary, Colon; Cervix
OD1	Nav1.3; Nav1.4; Nav1.6; Nav1.7 [126]	Prostate, Breast, Lung, Leukemia Mesothelioma Cervix, Ovary
To4	Nav1.1 to Nav1.7 [131]	Prostate, Breast, Lung, Leukemia Mesothelioma Cervix, Ovary ; Colon
Ts5	Nav1.2 to Nav1.7 [124]	Prostate, Breast, Lung, Leukemia, Mesothelioma, Ovary, Colon;

be due to the regulation of integrin activity by Kv1 channels. Indeed Arcangeli and Becchetti (2006) have suggested the existence of cross-talk between cell surface integrins and K^+ channels [143]. They demonstrated that K^+ channels form protein complexes with integrins and can have a direct lateral interaction. This is the case of the Kv1.3 channel which constitutes a macromolecular complex with the β1 subunit of the integrin receptor. Kv channel-integrin interaction has also been demonstrated using Kv channel blockers that affected the interaction of the Kv1.3 channel with β1 integrin and inhibited integrin-mediated cell adhesion to the extracellular matrix [144].

Margatoxin (MgTx), a Kv1.3 blocker from *Centruroides margaritatus* venom, significantly inhibited human lung adenocarcinoma cell proliferation and decreased tumor volume in mice [145]. Inhibition of Kv1.3 increased the expression level of p21Waf1/Cip1 along with decreased expression of Cdk4 and cyclin D3, to induce cell cycle arrest by blocking G1 to S transition [145]. The *in vivo* anti-tumor effect of MgTx was confirmed also in an orthotopic melanoma model using B16F10 cells [146]. Although different studies were focused mainly on the implication of Kv1.3 in regulating the cells proliferation, several reports suggested that the mechanism can be also extrapolated for other Kv channel subtypes [147].

On the other hand, the calcium-activated potassium channels (KCa) could also be implicated since the Iberiotoxin (IbTx), a scorpion toxin active on big conductance calcium-activated potassium channel (BKCa), inhibited the proliferation of glioma cells, by blocking the cell cycle in phase S and inhibiting the synthesis of DNA [148]. It is known that the balance of the Ca^{2+} concentrations between the intracellular and the extracellular compartments must be ensured by the cell in order to maintain the activation of the downstream processes leading to cell proliferation mechanism. Kv and KCa channels are crucial actors in this process by regulating the concentrations of the intracellular K^+ ions in a way to preserve the driving forces of the Ca^{2+} entry to the cell [149]. On the other hand it has also been reported that Iberiotoxin (IbTx) and charybdotoxin (CTx), blockers of KCa channels, inhibit the proliferation and migration of glioma and melanoma cells, respectively [150,151]. CTx inhibited the migration of the transformed renal epithelial cells (MDCK-F) by inducing a disruption of cell volume, actin filaments and $[Ca^{2+}]_i$ [151]. The same effect is obtained with LPA-stimulated migration of microglial cell [152]. Therefore, the inhibition of these channels by scorpion venom KTxs can have a drastic impact on the whole cancer cell hallmarks. In summary, the K^+ channel scorpion peptide blockers might target different cell signaling pathways (Fig. 7A). The effect on cell proliferation might in fact be the cause of the perturbation of the K^+ homeostasis related cell mechanisms that depend on the depolarization of the electrical potential. But there are also other signaling pathways that are triggered by the interaction with the potassium channel implicating EGFR, integrins as well as actin filaments. There might be other affected targets by the scorpion peptides activity which could also interact physically with the toxins. This seems to be a coherent hypothesis given that some peptides sharing high degree of sequence identity or having the same ion channel targets but with different levels of activities show different effects on different cancer hallmarks. More attention and rigorous investigations should be undergone to investigate these interactions and to establish the relationship between the scorpion peptide, the physically interacting receptors and the signaling pathways triggered by these interactions (Fig. 7A).

The identification of the interaction hotspots on the surface of K^+ scorpion peptide blockers has been supported by structural analysis and targeted chemical modification. The grouping of the basic residues on one surface side and acid residues on the opposite one of the toxin structure, defines its amphiphilic character. This heterogeneous distribution, or anisotropy of electrostatic charge, strongly polarizes the molecule and orients the toxin to its binding site [153].

The dipole moment vector, resulting from the anisotropy of electrostatic charge, emerges through the hotspot residue patch. For example, the dipole moment calculated for P05 and LTX emerges from the surface carried by the helix, while for ChTx and KTx it emerges from the surface carried by the beta sheet [154].

The interface is trained by the functional dyad and other critical residues. This dyad consists typically of a lysine and a hydrophobic residue (generally a Tyr, Phe or Leu), separated by a distance of 6–7 Å [20,155,156]. The functional dyad was identified in many toxins and it is generally present independently of the peptide structure. The conservation of the structure of the scorpion toxin and that of the functional dyad suggested that the peptide physically occludes the potassium channel pore by engaging the side chain of the lysine residue which occupies the binding site of the K^+ ions at the extracellular opening of the gate [155] (Fig. 7B). This has been confirmed by number of solid state NMR complexes and co-crystal structures [157,158].

3.3. Targets of chloride channel scorpion toxins in cancer cells

The chlorotoxin (ClTx), was the first successful example of anti-tumor scorpion toxin. This neurotoxin reduces the expression of ClC-3channel in glioma cells and delays the migration and invasion of tumor cells *in vitro* and *in vivo* [159,160] by decreasing the cellular volume [63,161]. ClTx crosses the hemato-encephalic barriers and binds to the malignant tumor cells of the brain without affecting healthy tissues [162]. *in vivo* assays have already shown that the iodine 125 and 131 labeled chlorotoxin (Chlorotoxin-$^{131}I/^{125}I$), have the capacity to specifically bind tumor brain cells, making this peptide a promising candidate in radiotherapy treatment of the post-surgical brain tumors [163,164], as well as loins and stomach tumors [165]. ClTx-conjugated nanoparticles are also used as carrier system to load anticancer drugs or therapeutic genes for targeted chemotherapy or gene therapy of glioma, respectively [166]. Furthermore, ClTx favors the efficiency and increases the bioavailability of certain clinical chemotherapies such as doxorubicin [167] and cisplatin [168]. Since 2007, the synthetic version of the chlorotoxin reached phase III of the clinical trials, under the name of TM-601 [152].

Many studies showed the potential therapeutic effect of other chloride channel scorpion toxins on gliomas. The rBmK-CTa (or Bm12b) inhibited the growth of the human glioma cells (SHG-44) in a dose-dependent manner with an IC_{50} of 0.28 μM. In the same conditions, the IC_{50} for normal astrocytes increases until 8 μM, indicating clearly the specific effect of the rBmK-CTa against the glioma cells [34]. BmK-CT (Bm12) and Gst-BmKCT also reduced the conductance of Cl^-current and the metastasis of glioma cells [31,169]. The synthetic and native AaCTx from *Androctonus australis* scorpion venom inhibited the invasion and the migration of U87 human glioma cells with IC_{50} of 10 μM and 125 μM, respectively [11]. This peptide presents 70% of sequence similarity with ClTx.

This scorpion toxins family, thus contains peptides with different effects on glioma cells. ClTx and AaCtx are active on their migration and invasion while rBmK-CTa inhibits their proliferation.

Studies made on the interaction of ClTx with glioma cells suggested that the receptor for ClTx seems to be a complex of proteins which contains the matrix metalloproteinase-2 (MMP-2) and the chloride channel ClC-3 [170]. The binding of ClTx to the tumor cell surface leads the endocytosis of this complex [171] which explains the irreversible action of this peptide and its relatively slow kinetic mechanism of action [172]. MMP-2 is implicated in the ECM degradation while CLC-3 is implicated in the cell volume shrinkage mechanism [172]. MMP-2 and CLC-3 channel were proposed as main macromolecular targets for the chloride channel scorpion peptide blockers [173] (Fig. 8A). Other studies showed that ClTx interacted with the MMP-2 isoform and not with MMP-1, 3 and 9 [170,174]. While the co-localization of MMP-2 and CLC-3 is confirmed [175], whether there is a physical interaction or a signaling cross-talk between the two targets remains an open question.

ClTx has a double activity on MMP-2. It reduced the expression of the protein on the cell surface via endocytosis and inhibited its

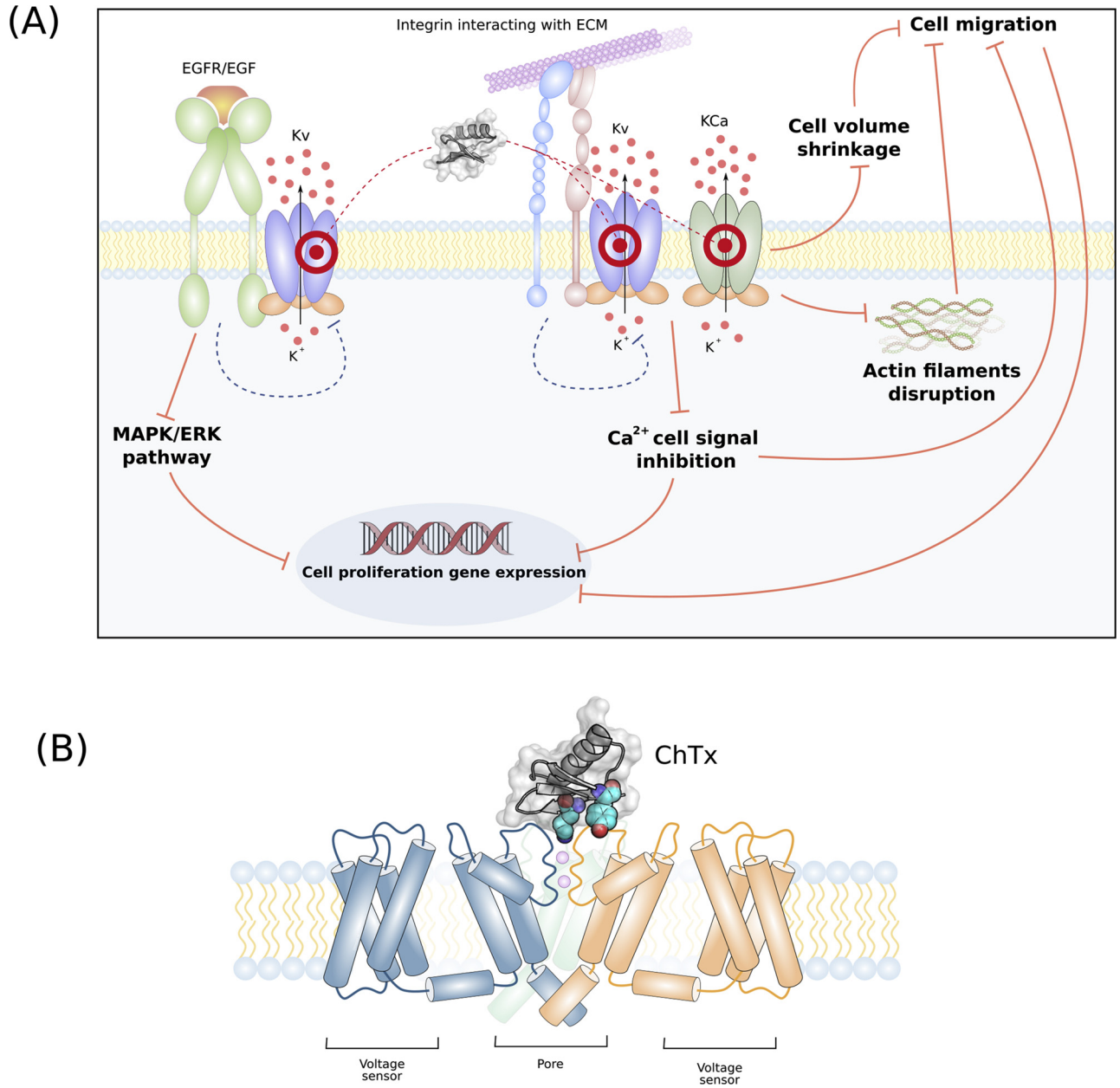

Fig. 7. Inhibition of cellular targets involved in cancer cell proliferation and migration mechanisms by KTx.
(A) Mechanisms of action of scorpion peptide blockers of voltage gated (Kv) or calcium activated (KCa) potassium channels, inhibiting cancer cell progression. Anti proliferative activity involves the inhibition of the outward K^+ flux which in turn affects the Ca^{2+} signaling. Other pathways may involve the interaction of EGFR and integrins with the potassium channel inhibited by the scorpion peptides. The disruption of cell volume shrinkage, actin filaments and $[Ca^{2+}]_i$ inhibit cell migration. Arrow headed and bar-headed lines indicate activation and inhibition relationship, respectively. The Blue bar-headed bar represent an auto-regulation mechanism by the complex formation of EGFR/Kv and Integrin/Kv. (B) mechanism the physical occlusion of the K^+ current by a scorpion peptide (Charybdotoxin).

enzymatic activity [170,176]. *In silico* analysis, suggested the implication of non-competitive inhibition mechanism of ClTx on MMP-2 with the interaction site of the toxin being on the collagen binding domain rather than the catalytic domain. Such model was able to explain several observations supported by previous experimental findings notably the interaction of ClTx with a supramolecular complex that includes MMP-2 among other macromolecules [177] (Fig. 8B).

Once activated, MMP-2 is recruited on the surface of the tumor cell invadopodia by the interaction with other molecules including integrins [178]. The discovery that MMP-9 is capable to cleave the extracellular S5-S6 loop of DII domain of Nav1.7 to alter pain signaling set a new question on a similar mechanism that can be found to regulate the cancer progression and the communication between different hallmarks of cancer [179]. Indeed, two derivatives of ClTx, CA4, and CTX-23, were found to inhibit the proliferation of glioma cell lines while no cation channel was identified as one of their targets [173]. These peptides are also effective in blocking endothelial tube formation and reduce tumor angiogenesis *ex vivo* [173].

3.4. Targets of calcium channels scorpion toxins in cancer cells

IpTxA, MCa, HCa and HdCa toxins were shown to modulate the activity of the RyR1 channel in a similar way to the synthetic A domain peptide of the II-III loop of Cav1.1 channel [37,38,180,181]. Thus the interaction “IpTxA-RyR”, “MCa-RyR”, “HCa-RyR” or “HdCa-RyR” mimics the molecular characteristics of the “II-III-RyR loop” interaction,

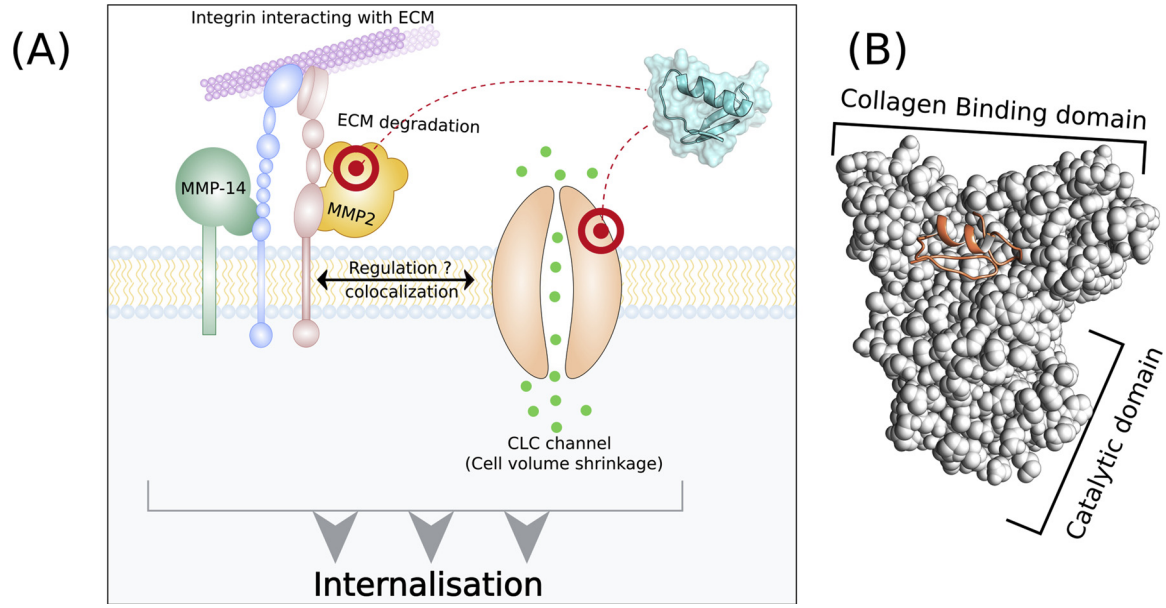

Fig. 8. Inhibition of cellular targets involved in cancer cell invasion and migration mechanism by chloride channels scorpion toxins.
(A) Signaling pathways involved in the mechanism of action of the scorpion peptide inhibiting MMP-2 and chloride channels. The anti-invasion of the scorpion peptides is presented at three levels: (i) The inhibition of the MMP-2 which limits the capacity of the cancer cell to degrade the ECM macromolecules, (ii) The inhibition of CLC channels which has an effect on the shrinkage capacity of the cell and (iii) the decrease of the expression the supra-molecular complex that incorporates MMP-2 and CLCs among other receptors. (B) Interaction of ClTx with MMP-2 describes a non-competitive inhibition of the protein but rather an interaction with the collagen binding domain.

despite the low similarity of sequences (˜18%) and structures between the toxins and peptide A.

The effect of maurocalcine MCa and IptxA on the ryanodine receptor implies their penetration into the cell [182–184]. The study of Estève et al. [184], showed that the maurocalcin-biotinyl/streptavidine-cyanine3 (fluorescent indicator) complex was capable of traversing the plasma membrane of several cell types without the use of cellular energy or the involvement of an endocytosis mechanism [184,185]. This work suggests a passive and rapid translocation (one to two minutes) of maurocalcine. This property is similar to Cell-Penetrating peptides, which could translocate to cellular cytoplasm by interacting with phospholipids and creating reverse micelles. The conserved basic sequence KKCKRR in MCa as well as HCa and IpTxA toxins seems to play not only an important role in the activation of RyR1 channels, but also in the interaction with lipids membrane, which require a positively charged surface patch [186,187] (Fig. 5).

The cell penetration property of MCa leads to its application by the team of De Waard [188], as a drug carrier to overcome the resistance of cancer cells to chemotherapy and to enhance the effect of these molecules. They showed that MCa allowed delivering non-permeate doxorubicin, a cytotoxic anticancer drug, and overcoming resistance to chemotherapy in the cancer cell line MDA-MB231 [188]. Furthermore, platinum-Maurocalcine conjugate (Pt-1-DMCa) induces apoptosis of human glioblastoma cells by inducing intracellular oxidative stress, DNA damage and led to enhanced p53 phosphorylation and AKT and ERK dephosphorylation [189,190].

Although, the conjugation of MCa has shown to enhance the antitumor activity of some chemotherapeutics, no study validated the anticancer effect of any toxin of this family when used alone. However, many studies reported the involvement of calcium in the different hallmarks of cancer cells in particular in the deregulation of proliferation, apoptosis and migration [191,192]. The modulation of ion channel function and the alteration of cytoplasmic, reticular and mitochondrial calcium homeostasis have shown to play an important role in the tumor mechanism [193].

Several reports indicate that non-cation channels voltage-gated family TRP (Transient Receptor Potential) is a key player in calcium homeostasis [194,195]. About 30 members of this superfamily have been identified in mammals and were classified into six different families: TRPC (for Canonical), TRPV (for Vanilloid), TRPM (for Melalstatin), TRPML (for MucoLipin), TRPP (for Polycystin) and TRPA (for Ankirin) [196].

Interestingly, high expression level and involvement of some of these channels in several cancer cells were reported. For example, TRPV provides the cytoplasmic calcium required to activate signaling downstream of cancer cell proliferation [197]. TRPV2 [198,199] and TRPV6 [200–203] are involved in prostate and bladder cancers progression. TRPV6 channel is modulated by a peptide named SOR-C13, from the saliva of the Northern Short-tailed shrew (*Blarina brevicauda*) [204]. This peptide is actually used in trial to stabilize tumor size of patients with ovarian cancer or other cancers known to over express the TRPV6 channel [204]. The TRPM7 expression level and the formation of metastases were positively correlated in breast cancer [205]. TRPC6 was overexpressed in breast, liver, stomach and glioma cancers [206]. This channel as well as TRPC1 were shown to facilitate the secretion of angiogenic factors by tumor cells and the formation of new capillaries [192,206]. The expression of TRPC3 was increased in breast and ovarian tumors [207].

The study of the pharmacological role of these receptors in cancer cells was established by shRNA and siRNA knockdown gene silencing technology [208–210], or by using chemical activators or inhibitors such as capsaicin [211]. Few scorpion peptides modulating these calcium channels have been identified. Tetrapandine-2, from *Pandinus imperator*, is an inhibitor of TRPC3 in human embryonic kidney-293 cells [212]. BmP01 from *Mesobuthus martensii* induces pain by targeting TRPV1 channel [213]. However, the anti-tumor effect of these peptides was never investigated. Thus, in view of wealth of scorpion venoms, other toxins with specificity to different calcinoma (proteins regulating calcium ion exchange [191]) that could have potential effect on cancer cells growth, have to be identified.

3.5. Other scorpion venoms molecules affecting cancer hallmarks

Several scorpion venom proteins/peptides belonging to other

classes than ionic channel toxins showed anti tumor effects. These include proteases with strong proteolytic and gelatinolytic activities, which considerably decreased the growth of human lung adenocarcinoma (A549) cells [214]. A hyaluronidase, BmHYA1, from *Buthus martensii* scorpion venom, was shown to modulate the expression of CD44, a cell adhesion molecule aberrantly expressed in many breast tumors and implicated in the metastasis. It reduced the CD44v6 variant expression in MDA-MB-231 breast cancer cell [215,216]. BMK-CBP, a serine proteinase-like protein dose-dependently inhibited the proliferation of the MCF-7 cancer cell line [56].

Polypeptide extract from the scorpion venom (PESV), a group of partially purified polypeptides from the crude venom of BmK, inhibits cell proliferation and induce cell apoptosis of DU 145 human prostate cancer cells [217]. PESV inhibit also the neovascularization of Lewis lung carcinomas (LLC) [218], H22 hepatoma [219] and other angiogenesis-dependent tumor growth by decreasing the level of angiogenic factors in tumor microenvironment [218–221].

Scorpion venom phospholipases A2 (PLA2) inhibited angiogenesis [222–224] as well as adhesion, migration and invasion of tumor cells by interacting with integrins [224–226].

Native and recombinant phospholipases A2 Sm-PLGV, from the venom glands of scorpion Scorpio maurus, inhibit both in vitro and *in vivo* angiogenesis by targeting integrins α5β1 and αvβ3 [226]. Hemilipin and Hemilipin2, two heterodimeric phospholipase A2 (sPLA2) from *Hemiscorpius lepturus* scorpion venom, inhibit angiogenesis both *in vitro* and *in vivo*. Its antiangiogenic effects were independent from the enzymatic activity [222,223].

Bengalin, a high molecular weight protein from *Heterometrus bengalensis* venom, was shown to induce apoptosis of human leukemic cells without cytotoxicity to normal human lymphocytes. The damaged nuclei and DNA fragmentation were accompanied by decreased expression of heat shock protein (HSP) 70 and 90, activation of caspases 3 and9, and induced cleavage of poly (ADP-ribose) polymerase (PARP) [227,228].

Many peptides showed apoptotic effect against cancer cells via different pathways. Neopladine 1 and Neopladine2, from *Tityus discrepans* scorpion venom bind to the cell surface of invasive SKBR3 breast cancer cells, induce the expression of Fas ligand and trigger cell apoptosis [229]. BmKn-2, an antimicrobial peptide from the venom of scorpion *Mesobuthus martensii Karsch* [230], induced apoptosis of human oral cancer cells via a p53- dependent intrinsic pathway [231].

Recently, short peptides, RK and RK1 of 17 and 14 amino acids, purified from the venom of *Buthus occitanus tunetanus* exhibited antitumor activity against different tumor cells [232,233]. RK was able to inhibit the cell adhesion of glioblastoma (U87), melanoma (IGR39) and rat pheochromocytoma (PC12) cells to different extracellular matrix (ECM) by interacting with both α1β1 and αvβ3 integrins [232]. RK1 was able to inhibit cell proliferation, migration of U87 and IGR39 and strongly inhibited *ex-vivo* vascular growth in chicken chorioallantoic membrane (CAM) model [233]. It induced a marked reduction in the number of new capillaries and branching vessels in the CAM, without affecting pre-existing vessels. Furthermore, a decrease in the vascular density could be observed. Quantification shows that the total vessel length was reduced by 25%, 48% and 77% for 2 μM, 4 μM and 8 μM concentration, respectively, compared with the untreated conditions [233]. However, the mode of action of this peptide is not yet identified.

4. Conclusion

It has been reported that the application of targeted cancer therapy lead to the adaptation or resistance of tumor cells to chemotherapy. As a result, scorpion peptides with potentially less side effects than the classical chemotherapeutic drugs appear to be promising anticancer agents for the prevention and/or reduction of metastatic tumors. Peptide- and protein-based drugs have long been recognized as having distinct advantages over their small molecule chemical counterparts. Endogenous peptides are often more potent and specific to their *in vivo* receptors resulting in fewer adverse effects. Herein, this review demonstrates that scorpion venom peptides exert anticancer effects by interfering with different processes or hallmarks of tumor development. Scorpion toxins have different effects even when belonging to a same structural family. Some of them are active on only one stage of cancer cells development (proliferation, adhesion, invasion or migration), while others are active on several hallmarks of cancer cells.

Therefore, the use of different scorpion toxins with the capacity to exert anticancer effects through multiple mechanisms may be an alternative in clinical approaches. The natural origin of these toxins can be exploited to produce clinically useful agents, as well as diagnostic tools for cancer assessment. However, despite the intensive efforts, the use of venom components as therapeutic agents in clinic has been moderately successful.

Although a number of venom components have a promising effect on tumor development, further pre-clinical and clinical studies are required to prove the efficacy and safety of these anticancer peptides.

Based on the continual discovery of ionic channels in cancer cells, many scorpion toxins are under evaluation and scorpion venoms still contain potential therapeutic peptides that will be interesting to discover. Nevertheless, much more efforts should be made to extensively evaluate the anticancer effects of scorpion toxins and elucidate mechanisms that involve other cellular targets. It is however, necessary to focus on holistic approaches that are more convenient to tackle these sort of questions. High Throughput transcriptome and proteome analysis would therefore play a more important role in the future years to dress a more accurate picture about the mechanism of actions of ion channel blocker scorpion peptides.

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