Chapter 9

Snake Venom Peptides: Promising Molecules with Anti-Tumor Effects

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1. Introduction

Tumorigenesis and metastasis are two processes with inter-related mechanisms. These include tumor growth and angiogenesis, detachment of tumor cells from the primary tumor, followed by migration through the local connective tissue and penetration into the circulation (intravasation). Once in the blood stream, tumor cells interact with circulating blood cells, arrest in the microvasculature of target organs, then extravasate and secondary proliferate. During each of these steps, integrin-mediated adhesion, migration, proliferation and survival of tumor cells and angiogenic endothelial cells play crucial roles [1,2].

Integrins are a family of heterodimeric transmembrane receptors that mediate cell-cell and cell-extracellular matrix (ECM) interactions. These cell adhesion molecules are composed by non covalent association of α and β subunits. Although 18 α and 8 β subunits have been described, only 24 different combinations have been identified to date [3]. Specific integrin heterodimers preferentially bind distinct ECM proteins. The repertory of integrins present on a given cell dictates the extent to which that cell will adhere to and migrate on different matrices. Several integrins, among others αv and α5β1, recognize the RGD sequence on their respective ligands. Other adhesive sequences in ECM proteins have also been observed, including the EILDV and REDV sequences that are recognized by integrin α4β1 in an alternatively spliced form of fibronectin [3]. On ligation to the ECM, integrins cluster in the plane of the membrane and recruit various signalling and adaptor proteins to form structures known as focal adhesions [4].

Integrin expression can also vary considerably between normal and tumor tissue. Most notably, integrins αvβ3, α5β1 and αvβ6 are usually expressed at low or undetectable levels in most adult epithelia but can be highly up-regulated in some tumors. Expression levels of some integrins, such as α2β1, decrease in tumor cells; potentially increasing tumor cell dissemination [5]. The integrin αvβ3 is particularly important for tumor growth and

invasiveness [6]. The receptor plays a major role in neo-vessels formation, its expression being strongly up-regulated in endothelial cells and specifically required during angiogenesis stimulated by basic fibroblast growth factor (bFGF) and tumor necrosis factor-α [7,8]. αvβ3 is functionally involved in the malignant spread of various tumor cell types such as breast carcinoma, prostate carcinoma and melanoma, and supports tumor cell adhesion and migration through endothelium [9] and matrix proteins [10,1]. Blocking αvβ3 is therefore expected to have a broad impact in cancer therapy and diagnosis. In the last decade, several clinical trials evaluating the efficacy of αvβ3 blockers have led to encouraging results. Thus, MEDI-522 (Vitaxin), a humanized antibody derived from the mouse LM609 monoclonal antibody, was recently reported to give positive results in a phase II trial enrolling patients with stage IV metastatic melanoma [11]. Cilengitide is an inhibitor of both αvβ3 and αvβ5 integrins; it is currently being tested in phase II trials in patients with lung and prostate cancers [12] and in phase II and Phase III trials studying their role against glioblastoma are currently underway.

In addition to their role in tumor cells, integrins are also important for the host cellular response against cancer. Endothelial cells, fibroblasts, pericytes, bone marrow-derived cells, inflammatory cells and platelets all use integrins for various functions, including angiogenesis, desmoplasia and immune response.

Nature has been a source of medicinal products for thousands of years among which snake venoms form a rich source of bioactive molecules such as peptides, proteins and enzymes with important pharmacological activities. International research and development in this area, based on multidisciplinary approaches including molecular screening, proteomics, genomics and pharmacological *in vitro*, *ex vivo* and *in vivo* assays, allow the identification and characterization of highly specific molecules from snake venom that can potently inhibit integrin functions. These anti-adhesive snake venom proteins belong to different families (phospholipases, disintegrins, C-type lectins and metalloproteinases). By targeting integrins, they exhibit various pharmacological activities such as anti-tumor, anti-angiogenic and/or pro-apoptotic effects.

2. Snake venom protein families

2.1. The Snake Venom Metalloproteinases (SVMP)

Metalloproteinases are among the most abundant toxins in many *Viperidae* venoms. SVMPs are monozinc endopeptidases varying in size from 20 to 100 kDa. They are phylogenetically most closely related to the mammalian disintegrin and metalloproteinase (ADAM) family of proteins. SVMPs are grouped into several subclasses according to their domain organization [13, 14, 15]. P-I SVMPs are the simplest class of enzymes that contain only a metalloproteinase (M) domain. P-II SVMPs contain a M domain followed by a disintegrin (D) domain. P-III SVMPs contain M, disintegrin-like (D) and cysteine-rich (C) domains. Formally called P-IV, the heterotrimeric class of SVMPs that contain an additional snake C-type lectin-like (snaclec) domain [16] is now included in the P-III group as a subclass (P-IIId).

Most of the functional activities of SVMPs are associated with hemorrhage or the disruption of the hemostatic system, which are primarily mediated by the proteolytic activity of the M domain. SVMPs cause hemorrhage by disturbing the interactions between endothelial cells and the basement membrane through the degradation of endothelial cell membrane proteins (e.g., integrin, cadherin) and basement membrane components (e.g., fibronectin, laminin, nidogen, type IV collagen) [17]. Blood coagulation proteins (e.g., fibrinogen, factor X, prothrombin) are also targets of their proteolytic activities.

Echis carinatus venom contains the specific prothrombin activators, ecarin [18,19] and carinactivase [20]. Adamalysin II, a non-hemorrhagic P-I SVMP isolated from *Crotalus adamantus* venom, cleaves and inactivates serum proteinase inhibitors including antithrombin III [21]. Kaouthiagin, isolated from the venom of *Naja kaouthia* specifically binds and cleaves von Willebrand factor (vWF), resulting in loss of both the ristocetin-induced platelet aggregation and collagen-binding activity of vWF [22]. Additionally, a large number of the P-III SVMPs can inhibit platelet aggregation, thus enhancing the hemorrhagic state [23]. The hemorrhagic P-III SVMP jararhagin from the venom of *Bothrops jararaca* has been shown to degrade platelet collagen receptor $\alpha_2\beta_1$ integrin in addition to fibrinogen and vWF, resulting in the inhibition of platelet aggregation [24]. Other platelet receptors are also degraded by SVMPs. GPIbα is cleaved by kistomin; mocarhagin and crotalin [25-27], and GPVI is degraded by alborhagin, crotarhagin and kistomin [28,29].

In the other side, it was reported that several SVMPs inhibited integrin-mediated adhesion of cancer cells on ECM proteins (table 1). BaG, a dimeric PIII class of SVMP from *Bothrops alternatus* with inactivated enzymatic domain but intact D/C domain, has been reported to inhibit fibronectin-mediated K562 cell adhesion *via* α5β1 integrin [30].

Proteins	Snake	Integrins	Effects	References
VAP1, VAP2	*Crotalus atrox*	α3,α6,β1	Induce apoptosis of HUVEC	[31,36]
HV1	*Trimeruserus flavoviridis*	-	Inhibits adhesion of HUVEC and induces apoptosis	[32]
Halysase	*Gloydius halys*	α1β1;α5β1	Inhibits proliferation and Induces apoptosis of HUVEC	[33]
VLAIPs	*Vipera lebetina*	-	Inhibits proliferation and Induces apoptosis of HUVEC	[34]
Graminelysin	*Trimeresurus gramineus*	α1β1;α5β1	Inhibits proliferation and Induces apoptosis of HUVEC	[35]
BaG	*Bothrops alternatus*	α5β1	Inhibits adhesion of K562 cells	[30]
TSV-DM	*Trimeresurus stejnegeri*	-	Inhibits cell proliferation and induces transient cell morphologic changes of endothelial cells.	[113]

Table 1. SVMP affecting tumor cells

Several apoptosis-inducing proteins have been purified from hemorrhagic snake venom, such as VAP1 and VAP2 (*Crotalus atrox*), HV1 (*Trimeresurus flavoviridis*), halysase (*Gloydius halys*), and VLAIPs (*Vipera lebetina*) [31-34], graminelysin [35]. They are members of the SVMP and ADAM family and induce apoptosis of human umbilical vein endothelial cells (HUVECs) [31,36]. The detachment of endothelial cells and resulting apoptosis could be an additional mechanism for the disruption of normal hemostasis by SVMPs. TSV-DM a basic metalloproteinase from *Trimeresurus stejnegeri* venom inhibits cell proliferation and induces cell morphologic changes transiently of ECV304 cells. However, DNA fragmentation and DNA content analysis demonstrated that this metalloproteinase could not induce ECV304 cells apoptosis.

2.2. The disintegrins

Disintegrins are a family of non-enzymatic and low molecular weight proteins derived from viper venom [37-39]. They are able to inhibit platelet aggregation and interact with adhesion molecules in particular integrins in a dose-dependent manner. They have a K / RTS sequence which is known as the RGD adhesive loop [37-39]. Their primary structure shows a strong conservation in the arrangement of cysteines [38]. Most disintegrins represent the C-terminal domain of metalloproteinases PIIa, d and e classes and are released into the venom by proteolytic cleavage [40,37,38]. A minority of these proteins exist as D / C domains from the class of SVMPs PIIIb.

Disintegrins can be conveniently divided into five different groups according to their length and the number of disulfide bridges [41]. The first group includes short disintegrins, single polypeptide composed of 49 - 51 amino acids with four disulfide bridges. The second group comprises medium disintegrins containing about 70 amino acids and six disulfide bridges. The third group includes long disintegrins of 83 residues linked by seven disulfide bridges. The disintegrin domains of PIII snake-venom metalloproteinases, containing approx. hundred amino acids with 16 Cysteine residues involved in the formation of eight disulfide bonds, constitute the fourth subgroup of the disintegrin family. Unlike short-, medium- and long-sized disintegrins, which are single-chain molecules**,** the fifth subgroup is composed of homo and heterodimers. The dimeric disintegrins subunits contain about 67 residues with four disulfide intra-chain bridges and two interchain bridges [42,43].

Although disintegrins are highly homologous, significant differences exist in their affinity and selectivity for integrins, which explains the multitude of effects of these molecules (Table 2).

Disintegrins were first identified as inhibitors of platelet aggregation and were subsequently shown to antagonize fibrinogen binding to platelet integrin αIIbβ3 [44,45]. After that, studies on disintegrins have revealed new uses in the diagnosis of cardiovascular diseases and the design of therapeutic agents in arterial thrombosis, osteoporosis, and angiogenesis-related tumor growth and metastasis (table 2). Triflavin from *Trimeresurus flavoviridis* venom was one of the first RGD-disintegrins shown to inhibit angiogenesis both *in vitro* and *in vivo*

[46]. Triflavin strongly inhibited cell migration toward vitronectin and fibronectin nearly thirty orders of magnitude greater than anti-αvβ3 monoclonal antibodies [46]. Triflavin was also more effective in inhibiting TNF-α-induced angiogenesis in the chicken chorioallantoic membrane (CAM) assay. Similar results were obtained with another RGD-disintegrin, rhodostomin, from *Agkistrodon rhodostoma* venom, which inhibits endothelial cell migration, invasion and tube formation induced by bFGF in Matrigel™ both *in vitro* and *in vivo* [47]. Rhodostomin effects were inhibited by anti-αvβ3 but not by anti-αvβ5 antibodies, thus supporting the hypothesis that the effects of RGD-disintegrins are mediated by blockade of the vitronectin receptor.

Proteins	Snake	Integrins	Effects	References
Triflavin	*Trimeresurus flavoviridis*	α5β1,αvβ3, α3β1	Inhibits adhesion of tumor cells to matrix proteins, cell migration and angiogenesis *in vitro* and *in vivo*	[46]
Rhodostomin	*Agikistrodon rhodostoma*	αvβ3,αvβ5	Inhibits cell migration, invasion of endothelial cells; inhibits angiogenesis *in vivo* and *in vitro*	[47]
Contortrostatin	*Agkistrodon contortrix contortrix*	αvβ3,α5β1, αvβ5, αIIββ3	Blocks adhesion, migration invasion of different type of tumor cells	[48]
Lebestatin	*Macrovipera lebetina*	α1β1	Inhibits migration and angiogenesis	[56]
Accurhagin-C	*Agkistrodon acutus*	αvβ3	Prevents migration and invasion of endothelial cells; anti-angiogenic activity *in vitro* and *in vivo*; elicites anoïkis	[58]
Eristostatin	*Eritocophis macmahoni*	α4β1,other integrin not yet determined	Inhibits cell motility; no effect on cell proliferation or angiogenesis	[59,60]
DisBa-01	*Bothrops alternatus*	αvβ3	Anti-angiogenic and anti-metastatic effect on melanoma cells	[62]
Leberagin-C	*Macrovipera lebetina*	αvβ3	Inhibits cell adhesion of melanoma tumor cells	[114]
Accutin	*Agkistrodon acutus*	αvβ3	Inhibits angiogenesis *in vitro* and *in vivo*; induces apoptosis	[115]

Table 2. Effects of disintegrins on cancerous cells

Contortrostatin, a disintegrin isolated from the venom of the southern copperhead snake, exhibits anti-cancer activity in a variety of tumor cells [48-50]. It does not display cytotoxic activity *in vitro* nor animals upon injection. Contortrostatin inhibits adhesion, migration, invasion, metastatic and angiogenesis of tumor and endothelial cells mediated by $\alpha v\beta 3$,$\alpha 5\beta 1$ and $\alpha v\beta 5$ [48,50-54]. Recently, contortrostatin showed an additive inhibitory effect in combination with docetaxel on the growth of xenograft tumors derived from prostate cancer cells [55].

Lebestatin is an example of a non toxic KTS-disintegrin isolated from *Macrovipera lebetina* that inhibits migration and VEGF-induced *in vivo* angiogenesis [56]. The presence of a WGD motif in CC8, a heterodimeric disintegrin from *Echis carinatus*, increases its inhibitory effect on $\alpha v\beta 3$ and $\alpha 5\beta 1$ integrins [57].

There are few reports regarding the effects of ECD-disintegrins on endothelial cell migration. Acurhagin-C, dose-dependently blocked HUVEC migration toward a vitronectin-coated membrane. Furthermore, acurhagin-C elicited endothelial anoïkis *via* disruption of the $\alpha v\beta 3$/FAK/PI3K survival cascade and subsequent initiation of the procaspase-3 apoptotic signaling pathway [58].

Eristostatin, an RGD-disintegrin from *Eristocophis macmahoni* was tested on individual metastasis steps such as cell arrest, extravasation and migration [59]. Eristostatin treatment did not prevent tumor cell extravasation or migration [60]. However, it was shown later that eristostatin inhibited melanoma cell motility, an effect mediated by fibronectin-binding integrins [61]. Interestingly, this disintegrin, contrary to other RGD-disintegrins, did not inhibit angiogenesis, as stated before [61]. DisBa-01, a $\alpha v\beta 3$ integrin-blocking RGD-disintegrin, inhibits not only migration of endothelial cells *in vivo* [62] but also *in vitro* migratory ability of fibroblasts and two tumor cell lines.

Since integrin receptors are also quite indiscriminate as they support cell adhesion to several substrates, it seems highly reasonable that the general RGD-disintegrin scaffold of the integrin-binding motif could be employed as a prototype for drug design for new anti-metastatic therapies *via* blocking both tumor cell adhesion and tumor angiogenesis.

2.3. The snake venom phospholipases

Snake venom is one of the most abundant sources of secretory phospholipases A2 (PLA2), which are one of the potent molecules in snake venoms [63-65].

PLA2 (EC 3.1.1.4)—are enzymes that catalyze the hydrolysis of sn-2-acyl bond of sn-3-phospholipids, generating free fatty acids and lysophospholipids as products [66]. They are currently classified in 15 groups and many subgroups that include five distinct types of enzymes, namely secreted PLA2 (sPLA2), cytosolic PLA2 (cPLA2), Ca^{2+} independent PLA2s (iPLA2), platelet-activating factor acetyl-hydrolases (PAF-AH), lysosomal PLA2, and a recently identified adipose-specific PLA2 [65,67]. PLA2 are low molecular weight proteins with molecular masses ranging from 13-19 kDa that generally require Ca^{2+} for their activities

[69,70]. Snake venom sPLA2 are secreted enzymes belonging to only two groups that are based on their primary structure and disulfide bridge pattern [68,71,72]. Those of group I are similar to pancreatic sPLA2 present in mammals, were found in venom of *Elapidae* snakes, while group II PLA2s belong to the *Viperidae* and are similar to mammals nonpancreatic, inflammatory sPLA2s [73,74]. The group II can be subdivided mainly in two subgroups, depending on the residue at position 49 in the primary structure: Aspartic acid-49 PLA2s are enzymatically active, while Lysine 49 present low or no enzymatic activity [75]. There are other subgroups, such as Asparagine-49, Serine-49, Glutamine-49 and Arginine -49 [76-83]. Studies have found that catalytic activity is reduced or even abolished when an Aspartic acid of native PLA2 is replaced by another amino acid [80,84].

Despite a high identity of their amino acid sequences, sPLA2 exhibit a wide variety of pharmacological properties such as anticoagulant, haemolytic, neurotoxic, myotoxic, oedema-inducing, hemorrhagic, cytolytic, cardiotoxic, muscarinic inhibitor and antiplatelet activities [63,85-92].

Recently, PLA2s have been shown to possess anti-tumor and anti-angiogenic properties (Table 3). CC-PLA2-1 and CC-PLA2-2 from *Cerastes cerastes* viper are non-toxic and acidic proteins. They have high inhibitory effects on platelet aggregation and coagulation. In addition, CC-PLA2-1 and CC-PLA2-2 inhibit the adhesion of the human fibrosarcoma (HT1080) and melanoma (IGR39) cells to fibrinogen and fibronectin. In the same direction, CC-PLA2-1 and CC-PLA2-2 potently reduces HT1080 cell migration to fibrinogen and fibronectin with nearly similar IC_{50} values [93]. This anti-adhesive effect was due to the inhibition of $\alpha 5\beta 1$ and αv-containing integrins [94]. A recent report demonstrated that Bth-A-I, a non-toxic PLA2 isolated from *Bothrops jararacussu* venom display an anti-tumoral effect upon breast adenocarcinoma as well as upon human leukaemia T and Erlich ascetic tumor [95].

Proteins	Snake	Integrins	Effects	References
CCPLA2-1; CCPLA2-2	*Cerastes cerastes*	$\alpha 5\beta 1, \alpha v$	Inhibits migration and adhesion of fibrosarcoma and melanoma cells	[93,94]
Bth-A-I-PLA2	*Bothrops jararacussu*	-	Anti-tumor activity on adenocarcinoma and leukaemia cells	[95]
MVL-PLA2	*Macrovipera lebetina*	$\alpha 5\beta 1, \alpha v$	Inhibits adhesion and migration of human microvascular cells and inhibits angiogenesis *in vivo* and *in vitro*.	[96]
BP II	Prothobotrops flavoviridis	-	Induces cell death in human leukaemia cells	[97]

Table 3. PLA2s targeting tumor cells

MVL-PLA2 is a snake venom phospholipase purified from Macrovipera lebetina venom that inhibited adhesion and migration of human microvascular endothelial cells (HMEC-1) without being cytotoxic. Using Matrigel™ and chick chorioallantoic membrane assays, MVL-PLA2, as well as its catalytically inactivated form, significantly inhibited angiogenesis both *in vitr*o and *in vivo*. Also, the actin cytoskeleton and the distribution of $\alpha v\beta 3$ integrin, a critical regulator of angiogenesis and a major component of focal adhesions, were disturbed after MVL-PLA2 treatment. The enhancement of microtubule dynamics of HMEC-1 cells, in consequence of treatments by MVL-PLA2, may explain the alterations in the formation of focal adhesions, leading to inhibition of cell adhesion and migration [96].

A cell death activity was discovered in Lysine 49-PLA2 called BPII. It induces caspase-independent cell death in human leukaemia cells regardless of its depressed enzymatic activity [97].

2.4. The C-type lectins

The C-type lectins are abundant components of snake venom with various function. Typically, these proteins bind calcium and sugar residues. However, the C-type lectin like proteins from snake venom (termed actually snaclec) does not contain the classic calcium/sugar binding loop and have evolved to bind a wide range of physiologically important proteins and receptors [98].

Snaclecs have a basic heterodimeric structure with two subunits, nearly always linked covalently, *via* a disulphide bond. The heterodimers are often further multimerized either non-covalently or covalently *via* additional disulphide bonds, to form larger structures [99]. The two subunits form a concave surface between them [100] thus constituting the main site of ligand binding [101,102]. The subunits have a high structural degree of homology between them and with other snaclecs [103]. Despite their highly conserved primary structure, the snaclecs are characterized by various biological activities. They were and are still considered as modulators of platelet aggregation by targeting vWF, GPIb-IX-V, GPVI and possibly other platelet receptors.

Recently, novel activities of snaclecs were highlighted. They were described for their potential anti-tumor effect by blocking adhesion, migration, proliferation and invasion of different cancer cell lines (Table 4). Among these proteins, EMS16, a heterodimer isolated from the venom of *Echis multisquamatus*, inhibits the adhesion of HUVECs cells on ECM proteins and their migration by inhibiting the binding of integrin $\alpha 2\beta 1$ to collagen [104].

Lebecetin and lebectin, purified from *Macrovipera lebetina* venom, are the only snaclecs, until today, with an evident anti-tumor effect in addition to their anti-aggregation activity on platelets. Indeed, these two non cytotoxic proteins inhibit the adhesion of various cancer cell lines: melanoma (IGR39), adenocarcinoma (HT29-D4), fibrosarcoma (HT1080) and leukemia cells (K562) on different ECM proteins. They also inhibit the proliferation, migration and invasion of HT1080 cells [105,106]. Lebectin also displays anti-angiogenic activity at very low concentrations both *in vitro* and *in vivo* [107]. Thus, lebectin presents the best anti-

angiogenic efficacy yet described for snake venom-derived peptides [108,109]. These observed effects are mediated by $\alpha 5\beta 1$ and αv integrins [107].

Extensive researches have been shown that cell adhesion activities in cancer disease are deregulated. According to this idea, it was also reported that lebectin inhibits these alterations by promoting N-cadherin/catenin complex reorganisation at cell-cell contacts, inducing a strengthening of intercellular adhesion [110].

Another snaclec, BJcuL isolated from *Bothrops jararacussa* venom, was also described for its anti-tumor, but the receptor or integrin implicated has not been determined yet. This homodimeric protein inhibits proliferation of several cell lines of renal, pancreatic, prostate and melanoma origin, but no effect was observed on colon or breast cancer cells [111]. BJcuL also affects the viability of some tumor cell lines of different origins, but has no effect on the growth of K562 and T24 cells, suggesting that these cells do not express the receptor recognized by the lectin. BJcuL induces apoptosis in human gastric carcinoma cells accompanied by inhibition of cell adhesion and actin cytoskeleton disassembly [112].

Proteins	Snake	Integrins	Effects	References
Lebecetin, lebectin	*Macrovipera lebetina*	$\alpha 5\beta 1$,αv	Inhibits adhesion, migration and invasion of human tumor cells; inhibits angiogenesis	[106]
BJcuL	*Bothrops jararacussu*	–	Inhibits tumor cell and endothelial cell growth; induces apoptosis of human gastric carcinoma cells; inhibits cell adhesion and actin cytoskeleton disassembly	[111,112]
EM16	*Echis multisquamatus*	$\alpha 2\beta 1$	Inhibits adhesion and migration of HUVEC cells	[104]

Table 4. Snaclecs and their effects on tumor cells

3. Potential application of snake venom compounds

Venoms are a rich source of molecules endowed with diverse pharmacological effects. Most part of these molecules act *via* the adhesion molecules. The intervention of the scientists and the clinicians in the pharmaceutical development field would employ these molecules as therapeutic agents for several pathologies such as cancer, thrombosis, diabetes....

Until now, no medicine was produced from a native molecule purified from venom. However, several peptidomimetics were designed by basing on the structure of these molecules. The benefits of these peptidomimetics compared to antibodies that can be used for the treatment of certain diseases are: a shorter half-life, reversible inhibition, easier to control a problem and very low immunogenicity. For example, the antihypertensive drug captopril, modelled from the venom of the Brazilian arrowhead viper (*Bothrops jaracusa*); the anticoagulant Integrilin (eptifibatide), a heptapeptide derived from a protein found in the

venom of the American southeastern pygmy rattlesnake (*Sistrurus miliarius barbouri*); Ancrod, a compound isolated from the venom of the Malaysian pit viper (*Agkistrodon rhodostoma*) for use in the treatment of heparin-induced thrombocytopenia and stroke and alfimeprase, a novel fibrinolytic metalloproteinase for thrombolysis derived from southern copperhead snake (*Agkistrodon contortrix contortrix*) venom (Table 5). Two venom proteins from the Australian brown snake, *Pseudonaja textilis*, are currently in development as human therapeutics (QRxPharma). The first is a single agent procoagulant that is a homolog of mammalian Factor Xa prothrombin activator, whereas the other is a plasmin inhibitor, named Textilinin-1, with antihemorrhagic properties.

Name	Snake	Target and function/treatment	Clinical stage
Capoten ® (Captropil)	*Bothrops jaracusa*	Angiotensin converted enzyme (ACE) inhibitor/ high blood pressure	Granted FDA approval
Integrilin ® (Eptifibatide)	*Sisturus miliarus barbouri*	Platelet aggregation inhibitor/acute coronary syndrome	Granted FDA approval
Aggrastat ® (tirofiban)	*Echis carinatus*	GPIIb-IIIa inhibitor/ myocardial infarct, refractory ischemia	Approved for use with heparin and aspirin for the treatment of acute coronary syndrome
Exanta	*Cobra*	Thrombin inhibitor/ arterial fibrillation and blood	Seeking FDA approval
Alfimeprase	(Agkistrodon contortrix contortrix)	Thrombolytic/ Acute ischemic stroke, acute peripheral arterial occlusion	Phase III
Ancrod ® (viprinex)	*Agkistrodon rhodostoma*	Fibrinogen inhibitor/ stroke	Phase III
hemocoagulase	*Bothrops atrox*	Thrombin-like effect and thromboplastin activity/ prevention and treatment of haemorrhage	Phase III
Protac/ Protein C activator	*Agkistrodon contortrix contortrix*	Protein C activator/clinical diagnosis of haemostatic disorder	Granted FDA approval
Reptilase	*Bothrops jaraca*	Diagnosis of blood coagulation disorder	Granted FDA approval
Ecarin	*Echis carinatus*	Prothrombin activator/ diagnostic	Granted FDA approval

Table 5. Drugs and clinical diagnostic kits from snake venom

Actually, most of the current anticancer therapies (radiotherapy, chemotherapy) are not specific and are targeting at both tumor cells and healthy cells. However, in recent years, new treatments tend to focus on the tumor microenvironment and particularly on the inhibition of tumor angiogenesis. These treatments are based on several active and non toxic proteins from snake venom, as for example contortrostatin from *Agkistrodon contortrix contortrix* and eristostatin from *Eristocophis macmahoo.* Although all these molecules are still currently in clinical trials, they could in the future open new ways of healing and could be used as drugs.

4. Conclusions

From the initial discovery of captopril, the first oral ACE inhibitor, to the recent application of disintegrins for the potential treatment of cancer, the various components of snake venoms have never failed to reveal amazing new properties. While the original native snake venom compounds are usually unsuitable as therapeutics, interventions by medicinal chemists as well as scientists and clinicians in pharmaceutical R&D have made it possible to use the snake venom proteins as potential drugs for multiple disorders or scaffolds for drug design.

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