Snake Venom Disintegrins: Natural Products with Antitumor Effect

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Authors' contributions

This work was carried out in collaboration between all authors. Author IM designed the review and wrote the first draft of the manuscript. Author DGK managed the literature searches, clinical information and case reports, author EM collected the data, mentored and was responsible for the final form of the article. All authors read and approved the final manuscript.

Mini-review Article

ABSTRACT

Cancer is a leading cause of disease globally. Treatment methods combine radiation therapy, surgery, novel drug chemotherapy and recently, nano drugs. During the past few years, the venoms of various vipers that selectively block the function of integrin receptors have been employed as potential drugs to fight cancer by inhibiting tumor progression. Special attention has been paid to disintegrins which represent an important constituent of viper venoms. Recent developments in evaluating disintegrin applications in *therapies* are discussed as well as the potential of using disintegrins in the future as antitumor agents.

1. INTRODUCTION

According to incidence statistics for cancer worldwide, there is an increasing rate of cancer cases around the world. It is predicted there will be 23.6 million new cancer cases worldwide each year by 2030, if recent trends in incidence of major cancers continue to rise [1,2].

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Suggestions have been made for synthesizing effective and more safe as well as human friendly anticancer agents [3,4] or re-examining existing drugs as potential future anticancer agents [5]. Cancer is characterized by an accelerated and uncontrolled multiplication of transformed, aberrant cells which lose their apoptotic ability [6]. It is a multigenic and multicellular disease that can arise from almost all cell types and organs with multifactorial etiology. There are many reasons of the increasing number of cancer patients and attempts have been made to describe various factors (modernizations) responsible for producing different types of cancers in humans [7-9].

Various therapies such as chemotherapy, radiotherapy, surgery and immunotherapy have been used to treat cancer, but chemotherapy remains the most prevalent option. The drawback of this approach is that many patients become resistant after some time [10]. Therefore new approaches have been considered such as the use of natural products and nanoparticle based drugs that seem to be quite effective providing low side effects and targeted action on cancer cells [11-13].

Disintegrins have increasingly been used, especially those derived from vipers as anti-tumor agents exhibiting a particular affinity for integrins and appearing to represent a potential option for fighting cancer cells [14].

2. INTEGRINS AND CANCER

Integrins play a major role in many steps of the carcinogenesis progression. They are dimeric adhesion receptors that mediate cellular attachment to the extracellular matrix (ECM) or to adjacent cells. All integrins are alpha/beta ($α/β$) heterodimers and various pairings of α and β subunits confer to specificity towards distinct ECM proteins. Different α subunits may combine with the same β subunit and conversely different β subunits are capable of pairing with a particular α subunit [15,16]. Their heterodimeric composition generally confers to ligand specificity, according to which they are classified in four classes, recognizing various motifs but mostly in general the sequence-Arg-Gly-Asp-(RGD) [17].

The binding of ECM components with integrins on the cell surface, activates integrins aggregation and interaction with cytoskel et al.

elements, resulting in the formation of focal adhesions [18]. The latter, when formed, trigger a series of intracellular signaling essential for cell adhesion, spreading, migration, proliferation, survival and differentiation [19]. Several observations indicate that the changes in integrin activation state as well as the alteration in the level of expression of integrins or their ECM ligands, contribute to neoplastic progression [20]. For example, the level of expression of integrin alphavbeta3 ($α_Vβ3$), a vitronectin receptor that binds to extracellular matrix molecules via an RGD-binding site [21] in melanoma and other cancers, is proportional to the invasiveness of the tumors [22].

In breast cancer high expression of activated alphavbeta3-but not that of non-activated alphavbeta3-is associated with a highly aggressive metastatic phenotype, signalizing the onset of a widespread metastasis [23]. Since the RGD peptide sequence is an important cell attachment recognition site for several classes of integrins in many ECM proteins, agents that disrupt interactions of these integrins should have significant anti-tumor activity. Among these are monoclonal antibodies, peptidomimetics, small molecule compounds [24] and disintegrins [25].

3. SNAKE VENOM DISINTEGRINS

Snake venoms are complex mixtures of components with diverse pharmacological activity and toxicity [26]. Disintegrins were discovered and isolated from the venom of viper snakes and were given their name because of their biological function of binding to integrins [26]. Initial report in the literature, was 25 years ago, referring to trigramin [27]. Snake venom disintegrins consist a family of small (forty to one hundred amino acids) cysteine rich polypeptides [28], initially described as potent inhibitors of the platelet fibrinogen receptor, integrin $α_{IIb}β_3$ [27]. They are mostly derived from snake venom hemorrhagic metalloproteinases (SVMPs) [29] and members of this protein family have been classified according to their multi-domain structure into three classes [30]. P-I metalloproteinases (25-30 kDa) are only single-domain proteins, while P-II metalloproteinases (30-45 kDa) contain a disintegrin domain at the carboxyl terminal of a metalloproteinase domain structurally similar to that in the class P-I [31]. Hemorrhagins of the P-III class are large toxins (60-100 kDa) and compromise multi-domain enzymes that are built up by an N-terminal

metalloproteinase domain and a C-terminal disintegrin-like and cysteine-rich domains [32,33]. Disintegrins and disintegrin-like domains are released in the venoms by proteolytic processing of PII and PIII metalloproteinases [34].

Currently, snake venom disintegrins can be divided into subfamilies according to their structure, as well as their function [35]. Structurally, they can be divided into monomers and dimers in homodimeric and heterodimeric forms. Monomeric disintegrins are grouped according to their polypeptide chain length and the number of cysteines: Short disintegrins contain 8 cysteines, medium ones 12 cysteines and long disintegrins contain 14 cysteines. Each subunit of dimeric disintegrins contains 10 cysteines. Functional classification of snake venom disintegrins was determined by a presence of tripeptide motif in the active side. Currently, three functional groups of disintegrins may be distinguished, which express RGD-, MLD-and KTS-sequences [36].

Most of them are RGD-disintegrins binding to aIIbb3 integrin (fibrinogen receptors), avb3 integrin (vitronectin receptors) and a5b1 (fibronectin receptors). MLD-disintegrins are heterodimeric and bind to of a4b1, a4b7 and a9b1 integrins. The presence of another motif in the second subunit such as RGD, determines their activity towards other integrins including α5β1. KTS-disintegrins structurally are short monomeric molecules, which selectively bind to the collagen receptor, a1b1 integrin [36].

Disintegrins from different snake species have been isolated: Albolabrin, applagin, barbourin, batroxostatin, bitistatin, obtustatin, schistatin, echistatin, elegantin, eristicophin, flavoridin, halysin, kistrin, tergeminin, salmosin, triflavin and contortrostatin. These molecules are currently used as experimental therapeutic agents against a number of pathologies including Alzheimer's disease, inflammation, autoimmune diseases, virus infection, asthma, osteoporosis, thrombosis and cancer [26,37].

4. DISINTEGRINS AS ANTI-TUMOR AGENTS

Many approaching methods have been used in order to investigate the effect of different RGD disintegrins on various cancer cells. Table 1 summarizes the effects of various disintegrins on migration-dependent tumor cell activities. Contortrostatin (CN), a homodimeric snake venom disintegrin which mainly binds to integrin avb3 and avb5, was tested on different tumor types. A major report showed that contortrostatin inhibits growth and metastasis of MDA-MB-435 tumor masses in an orthotopic xenograft nude mouse model [38]. Also, CN has been reported to inhibit ovarian cancer dissemination in a nude mouse model as well as preventing the recruitment of blood vessels to tumors at secondary sites [39] while inhibiting tumor growth, angiogenesis and prolonging survival in a rodent glioma model [40]. In addition, this disintegrin seriously affects morphology, adhesion and mobility and induces breast cancer cells of primary cultures to apoptosis [41]. Cell culture experiments of PC-3 cells indicate that the combination of docetaxel-a chemotherapeutic drug-and CN inhibits cell growth in an additive fashion [42]. A short monomeric disintegrin eristostatin has been found to inhibit melanoma cell metastasis due to interference with $α_4β_1$-VCAM binding in addition to the inhibition of the classical RGD-binding integrins [43]. Furthermore eristostatin has been shown to inhibit melanoma cell migration on fibronectin in a concentration-dependent manner, but not that on collagen IV or laminin. In contrast, this disintegrin was found to have no effect on cell proliferation or angiogenesis [44]. Studies done with melanoma cells on a culture dish and natural killer cells attached to a cantilever tip in atomic force microscopy showed four major populations of interactions which exhibited altered frequency and unbinding strength in the presence of eristostatin [45]. Albolabrin is the primary published disintegrin which has been found to inhibit the attachment of B16-F10 mouse melanoma cells to either fibronectin or laminin absorbed on plastic [46]. Another monomeric disintegrin triflavin inhibits tumor cell-induced platelet aggregation [47] a result that is also achieved with the use of ussuristatin 1 [48]. Rhodostomin has been demonstrated to inhibit cell adhesion, migration and invasion of both breast and prostate carcinoma cells [49].

MLD-disintegrins generally interact with α4β1, α4β7 and α9β1 integrins. Especially VLO5 disintegrin binds to α9bβ1 integrin and inhibits tumor growth and induce to apoptosis in glioblastoma LN229 cancer cells [50]. Also this heterodimeric disintegrin acts as an antitumor agent in another type of brain cancer, medulloblastoma [51]. Tumor growth in lung cancer cells is also suppressed by VLO5 disintegrin [52].

Table 1. Types of disintegrins, structural determinants, preferential integrin, origin of disintegrins and relevant inhibitory activity

Disintegrin	Structure	Adhesive motif	Preferred integrin	Source (snake species)	Relevant inhibitory activity	Ref.
Rodostomin	monomeric short	RGD	avb3	*Calloselasma rhodostoma*	tumor angiogenesis	[49]
Albolabrin	monomeric medium	RGD		*Trimeresurus albolabris*	melanoma metastasis	[46]
Triflavin	monomeric short	RGD	avb3	*Trimeresurus flavoviridis*	angiogenesis	[47]
Trigramin	monomeric medium	RGD	avb3	*Trimeresurus gramineus*	bone metastasis	[27,58]
Eristostatin	monomeric medium	RGD	$a_{II}B3$	*Eristicophis macmahoni*	melanoma metastasis	[43]
Echistatin	monomeric medium	RGD	avb3	*Echis carinatus*	osteoclast migration	[37,57]
Contortrostatin	monomeric medium	RGD	avb5, avb3	*Agistrodon contotrix*	tumor angiogenesis	[38]
Obtustatin	monomeric short	KTS	a1b1	*Vipera lebetina obtusa*	endothelial cell proliferation	[53]
Viperistatin	monomeric short	KTS	a1b1	*Vipera palestonae*	melanoma cell transmigration	[54]
Lebestatin	monomeric short	KTS	a1b1	*Macrovipera lebetina*	angiogenesis	[53]
VLO5	heterodimeric	VGD, MLD	a9b1	*Vipera lebetina optusa*	glioblastoma growth	[50]

A KTS-disintegrin, lebestatin interacts specifically with the alpha1beta1 integrin being therefore able to inhibit both adhesion and migration of PC12 and alpha1beta1 integrin-expressing CHO cells (CHO-alpha1) onto type I and IV collagens. Using the 8-day-old embryo chick chorioallantoic membrane model [53] this disintegrin has also been shown to disrupt adhesion and migration of endothelial cells exhibiting an anti-angiogenic effect in vivo as well.

Other KTS-disintegrins such as obtustatin and viperastatin also bind exclusively and inhibit the adhesion of a1b1 integrin to collagen IV. Obtustatin is able to decrease endothelial cell proliferation by inducing apoptosis via an extrinsic mechanism and inhibits growth factor induced migration in those cells [53]. Furthermore, viperistatin blocks adhesion of human melanoma cell line HS 939.T onto collagen type IV by binding to α1β1 integrin [54].

In summary, snake venom disintegrins have been extensively studied as far as their relevant inhibitory activity effect on cell migration is concerned. In relation to the toxic properties and inhibitory activity of disintegrins, experimental data show that greatly depend on preferred integrin target as well as cognate ligand [55]. Very low doses of injected disintegrins (0.6 μg/mouse) have been tested and found to be effective in several cases of antithrombotic therapy [56]. Daily local injection of contortrostatin (5 μg/mouse/day) into MDA-MB-435 tumor masses have been proved to inhibit growth of the tumor by 74% [38]. On the other hand, *in vitro* studies indicate that increasing doses of contortrostatin above 10 μg/ml induce cancer cells to enter apoptosis [41]. Finally, it is worth mentioning that chimeric recombinant disintegrins have been used in an effort to offer more effective therapies in phase II trials, using liposomal encapsulation for delivery to target tissues [59].

5. CONCLUSION

Disintegrins are natural product derived compounds with potential antitumor effect. They have been shown to inhibit cell adhesion by binding to different kinds of integrins and drive cell migration to a halt or force several types of cancer cells to enter apoptosis. RGD-disintegrins are the most popular, exhibiting a profound effect on several types of tumors. Among them, contortrostatin appears to be the choice for many investigators since it exhibits the most profound antitumor effect in many experimental animal models. MLD and KTS disintegrins are yet in early investigating stages and their action needs further examination. In conclusion, snake venom disintegrins appear to be quite attractive biomolecules for tumor progression fighting studies. They have already been tested extensively in *in vitro* studies and in experimental animals but much more work has to be done in order to ensure that the can be safely used in clinical trials to fight tumors. Their role in clinical trials has been debated in a number of diseases but their therapeutic use is still controversial [60].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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