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ARTÍCULO DE REVISIÓN

PERSPECTIVES ON METASTASIS AND SNAKE VENOMS

PERSPECTIVAS SOBRE METÁSTASIS Y VENENOS DE SERPIENTE

Jorge Gutiérrez-Pajares[1] Isabel Gómez-Bentancur[2]

[1] Universidad Le Cordon Bleu, Lima, Perú.
[2] Universidad de Antioquía, Medellín, Colombia

Correspondencia:
Jorge Gutiérrez-Pajares
jorge.gutierrez@ulcb.edu.pe

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ABSTRACT

Metastasis is the major responsible for the death of cancer patients. This malignant feature implies the acquisition of protein machineries that allow cells to migrate, modify the extracellular matrix and form new blood vessels. Since the discovery of disintegrins as ligands and blockers of integrins, many studies have demonstrated that snake venoms are a source of bioactive molecules with anti-metastatic effect. In this review, snake venom molecules are described as inhibitors of metastasis at different levels, pointing to a need for further research.

Keywords: metastasis, snake, cancer, venoms, disintegrins.

RESUMEN

La metástasis es la principal causa de muerte de los pacientes con cáncer. Esta característica maligna implica la adquisición de maquinarias proteicas que permitan a las células migrar, modificar la matriz extracelular y formar nuevos vasos sanguíneos. Desde el descubrimiento de las desintegrinas como ligandos y bloqueadores de las integrinas, numerosos estudios han demostrado que los venenos de serpiente son una fuente de moléculas bioactivas con efecto antimetastásico. En esta revisión, las moléculas de veneno de serpiente se describen como inhibidores de la metástasis en diferentes niveles, lo que indica la necesidad de realizar más investigaciones.

Palabras clave: metástasis, serpiente, cancer, venenos, desintegrinas.

INTRODUCTION

Cancer is a chronic disease triggered by the accumulation of mutations in key genes (Takeshima & Ushijima, 2019) that can be simply described as a continuous proliferation of cells carrying active tumor promoting genes. Progression of this disease manifests in allowing cancer cells to leave their primary site, using the blood vessels and colonizing distant tissues/organs (Fares et al., 2020). This malignant progression is known as metastasis and is responsible for more than 90 % of deaths caused by cancer (Chin et al., 2005; Mittal, 2018).

Despite the huge pharmacological effort to minimize the effects of metastasis, no effective treatment has been delineated due its complexity. More basic information is still required to design a combination therapy to target multiple pathways for a successful treatment (Fares et al., 2020). Here, a brief review of important metastasis related-topics is described as well as research on snake venom active compounds that could be applied for the inhibition of metastasis-related cancer cell abilities.

Metastasis

Upon malignant progression, cancer cells from solid tumors acquire novel mutation-driven features that allow them to abandon their original site, the primary tumor, reaching to other tissues, the metastatic sites. Cancer metastasis involves three major processes:

Migration/invasion

Cancer cell migration involves the polarization of the cytoskeleton to create a leading protrusion that interacts with the extracellular matrix (ECM) to generate force and direct cell movement. In the plasma membrane of the leading protrusion, ECM binding-integrin proteins are clustered (Yamaguchi & Condeelis, 2007). These integrins are specialized proteins. that transduce the extracellular adhesion to intracellular mechano-signaling (Hamidi & Ivaska, 2018). Then, the cellular contraction promotes the detachment of adhesion bonds at the trailing edge. Importantly, migrating cells require mesenchymal intermediate filaments such as vimentin to protect them from mechanical stress (Patteson et al., 2020).

Pro-tumorigenic integrins are increased in the plasma membrane of invasive cancer cells (Ramovs et al., 2021), increasing the intracellular signaling of focal adhesion kinase (FAK), Src and Akt, among others, to promote cancer cell motility (Cooper & Giancotti, 2019).

In addition to increased motility, there is a transcriptional activation of metalloproteinases in invading cancer cells (Zitka et al., 2010). These enzymes are important to modify the ECM and are required to invade tissues locally and at metastatic sites (Conlon & Murray, 2019).

Angiogenesis

Another relevant hallmark of cancer cells to promote metastasis is the induction of blood vessels by recruiting endothelial cells, named angiogenesis. Newly formed blood vessels within the tumor promotes the rapid expansion of its tumor mass and contributes to the dissemination of cancer cells. The requirement of nutrients and oxygen, drives cancer cells to stimulate the migration of endothelial cells towards the tumor (Yang et al., 2013). Several angiogenic factors have been described (Nishida et al., 2006). Among them, the vascular endothelial growth factor family has been intensively studied due to its potent effect as chemoattractant and stimulator of proliferation of endothelial cells (Nishida et al., 2006).

Epithelial-mesenchymal transition

The acquisition of mesenchymal characteristics

allows cancer cells to gain invasive and migratory features (Zhang et al., 2022). Normally, epithelial cells present cell to cell interactions that maintain their organized structure (Garcia et al., 2018). Once the EMT has been triggered, the epithelial joint proteins decrease and there is an up-regulation of mesenchymal cytoskeleton proteins, such as vimentin (Saentaweesuk et al., 2018). Multiple transcription factors control EMT. The most well-described EMT transcription factors are Twist, Snail, Slug and ZEB1/2 (Kang, Y., & Massagué, J., 2004; Li, M et al., 2017; Li, Y et al., 2021; Wang et al., 2013; Wels et al., 2011).

Snake venom and metastasis

Disintegrins and cancer migration/invasion

A group of venom molecules named disintegrins have shown a regulatory effect on metastasis-related features. Several disintegrins have been extensively studied (Table 1). Disintegrins are integrin ligands that prevent integrin-mediated ECM interaction through a tripeptide RGD motif (Chen et al., 2020). Mechanistically, disintegrins perturb the intracellular mechano-signaling molecules. Thus, it has been reported that the disintegrin contortrostatin binds to α(v)β3 integrin causing the activation of the cytoplasmic kinase Src and the hyperphosphorylation of the focal adhesion kinase to inhibit the motility of cancer cells (Ritter et al., 2000). On the other hand, echistatin was shown to decrease the phosphorylation and activity of FAK as well as to reduce paxillin phosphorylation altering focal adhesion organization and therefore affecting cell adhesion and motility of B16-BL6 mouse melanoma cells (Della Morte et al., 2000).

Interestingly, in addition to interfering with integrin function, some disintegrins may have other anti-tumorigenic effects. Thus, while testing the mechanisms of action of eristostatin *in vivo*, it was observed that this disintegrin failed to block extravasation and migration of mouse melanoma cells but inhibited cancer cell growth after extravasation (Morris et al., 1995).

Snake venom molecules and epithelial-mesenchymal transition (EMT)

In addition to disintegrins, other molecules present in snake venoms have been identified as blockers of the EMT. Agkihpin (Huang et al., 2016), isolated from *Gloydius halys Pallas*, and cardiotoxin III (Tsai et al., 2016), isolated from *Naja naja atra,* have been demonstrated to perturb the canonical Wnt/β-catenin pathway by increasing E-cadherin and decreasing N-cadherin and vimentin and the EMT drivers Snail and Twist in human hepatocarcinoma and breast cancer cells, respectively. A similar mechanism for regulating EMT has been described for the PLA2-BthTX-II from *Bothrops jararacussu* (de Vasconcelos Azevedo et al., 2019).

The ectopic expression of cystatin, identified in *Notechis scutatus scutatus*, in a human liver cancer cell line elevated the E-cadherin levels while decreasing the expression of N-cadherin to reduce the invasion and metastasis ability of these cells (Tang et al., 2011). Moreover, recombinant cystatin produced in yeast inhibited the invasion of both melanoma and liver cancer cells (Xie et al., 2011).

Snake venom molecules and angiogenesis

It has been described that the disintegrin salmosin inhibits tumor-specific angiogenic activity by blocking the vascular endothelial α(v) β3 integrin (Kang et al., 1999). In a similar way, contortrostatin inhibits angiogenesis of the MDA-MB-435 breast cancer cells in an *in vivo* model of tumor growth (Zhou et al., 2000). Rhodostomin also inhibits melanoma cells induced angiogenesis by affecting endothelial cells but not cancer cells (Yeh et al., 2001). The anti-angiogenic potential of the CC5 and CC8 disintegrins from *Cerastes cerastes* was also demonstrated in the embryo chick chorioallantoic membrane model and rat aortic ring assays (Ben et al., 2016). The metalloproteinase bothropoidin

TABLE 1

List of disintegrins present in snake venoms that have been reported to exert an anti-metastatic effect

Molecule	Isolated from	Anti-metastatic effect	Reference
Albolabris	*Trimeresurus albolabris*	Inhibitor of adhesion of mouse melanoma cells to fibronectin and laminin	(Soszka et al., 1991)
Alternagin-C	*Bothrops alternatus*	Decreases adhesion of breast cancer cells to collagen and increases the expression of the metastasis suppressor 1 and decreases MMP9 and -2 metalloproteinases	(Moritz et al., 2022)
Bothropoidin	*Bothrops pauloensis*	Inhibits the adhesion migration of human breast cancer cells	(Guimarães et al., 2017)
Colombistatin	*Bothrops colombiensis*	Inhibitor of human cancer urinary and melanoma cells to adhere to fibronectin and to migrate	(Sánchez et al., 2009)
Contortrostatin	*Agkistrodon contortrix*	Inhibitor of adhesion of human melanoma cells to type I collagen, vitronectin, and fibronectin via β1 integrin. Also, this molecule inhibits *in vivo* lung colonization.	(Trikha et al., 1994)
		Binds to αvβ5 integrin to inhibit invasion of the ovarian can cancer cells	(Zhou, Nakada, et al., 2000) (Markland et al., 2001)
		Diminishes more than 60 % of lung metastasis of breast cancer cells	(Zhou, Sherwin, et al., 2000)
		Inhibits adhesion of human glioblastoma cells to vitronectin and fibronectin	(Schmitmeier et al., 2000)
Dis-Ba-01	*Bothrops alternatus*	Inhibits the adhesion of mouse melanoma cells to vitronectin	(Ramos et al., 2008)
		Reduced migration speed and directionality of human oral squamous cancer cells via αvβ3 integrin	(Montenegro et al., 2017)
EC3	*Echis carinatus*	Inhibitor of adhesion of α4β1 and α4β7 integrins in human leukemia cells	(Marcinkiewicz et al., 1999)

Molecule	Isolated from	Anti-metastatic effect	Reference
Echistatin	*Echis carinatus*	Inhibitor of mouse melanoma cells adhesion to fibronectin, vitronectin and laminin	(Staiano et al., 1995)
		Blocks FAK and paxillin phosphorylation	(Della Morte et al., 2000)
Eristostatin	*Eristicophis macmahoni*	Inhibitor of human melanoma cell metastasis in murine model	(Danen et al., 1998)
Jararhagin	*Bothrops jararaca*	Decreased proliferation and adhesion properties of human melanoma cells	(Corrêa et al., 2002)
Lansbermin-I	*Porthidium lansbergii lansbergii*	Inhibited the adhesion and migration of human breast cancer cells	(Montealegre-Sánchez et al., 2019)
Lebectin	*Macrovipera lebetina*	Inhibited the in vitro migration of human fibrosarcoma cells seeded on fibronectin matrix.	(Sarray et al., 2004)
Obtustatin	*Vipera lebetina*	By inhibiting the α2β1 integrin diminished the murine Lewis lung carcinoma growth	(Marcinkiewicz et al., 2003)
r-Mojastin 1	*Crotalus scutulatus scutulatus*	Inhibits human urinary cancer cells to adhere to fibronectin	(S. Lucena et al., 2011)
		Inhibited the migration of human pancreatic carcinoma cells	(S. Lucena et al., 2015)
Rhodocetin	*Calloselasma rhodostoma*	Blocks fibrosarcoma cells via α2β1 integrin	(Eble et al., 2002)
Rhodostomin	*Calloselasma rhodostoma*	Inhibits the in vivo tumor growth of melanoma cells by reducing angiogenesis	(Yeh et al., 2001)
r-Rubistatin	*Crotalus ruber*	Inhibits the *in vitro* migration of melanoma cells	(Carey et al., 2012)
Salmosin	*Agkistrodon halys brevicaudus*	Recombinant salmosin blocks mouse melanoma cell invasion *in vitro* and prevents lung tumor colonization *in vivo*.	(I. C. Kang et al., 1999)
		Inhibited the adhesion of human melanoma cells to collagen and vitronectin	(Chung et al., 2003)
Saxatilin	*Gloydius halys*	Inhibited the tumor necrosis α-induced invasion of human ovarian cancer cells	(Kim et al., 2007)
		Blocked the pro-angiogenic effect of human lung cancer cells *in vitro*	(Jang et al., 2007)

Molecule	Isolated from	Anti-metastatic effect	Reference
Trigramin	*Trimeresursus stejnegeri*	Potent inhibitor of adhesion of human melanoma cells to fibronectin and fibrinogen	(Knudsen et al., 1988)
Triflavin	*Trimeresurus flavoviridis*	Inhibitor of adhesion of human cervical carcinoma cells to fibronectin, fibrinogen, and vitronectin	(Sheu et al., 1994)
Tzabcanin	*Crotalus simus tzabcan*	Inhibits migration of human melanoma and lung cancer cells via αvβ3 integrin	(Saviola et al., 2016)
Ussuristatin 1	*Agkistrodon ussuriensis*	Inhibitor of adhesion of human melanoma cells to fibrinogen and fibronectin	(Oshikawa & Terada, 1999)
Vaa-Dis	*Vipera ammodytes*	Inhibits migration of human breast cancer cells	(Latinović et al., 2017)
Viperstatin	*Vipera paleastinae*	Inhibits the adhesion of cancer cells expressing α1β1 or α2β1 to collagen types I and IV	(Staniszewska et al., 2009)
r-Viridistatin 2	*Crotalus viridis viridis*	Inhibits the invasion of bladder and breast cancer cells.	(S. E. Lucena et al., 2012)
		Inhibited the migration of human pancreatic carcinoma cells	(S. Lucena et al., 2015)

inhibits the basic fibroblast growth factor-induced tube formation of endothelial cells (Guimarães et al., 2017).

Pre-clinical studies

A few snake venom molecules have been assayed in *in vivo* experiments. The disintegrin contortrostatin has been tested in a liposomal delivery system to reduce the daily intratumor injection and increase its circulatory half-life maintaining its anti-angiogenic activity in a murine xenograft human breast tumor (Swenson et al., 2004). In another experimental model, the intravenous administration of the recombinant αvβ3 integrin binding disintegrin DisBa-01 inhibited lung metastasis of murine melanoma cells (Ramos et al., 2008). Similarly,the administration of recombinant cystatin prevented the colonization of murine melanoma cells to lungs in an autochthonous mouse *in vivo* metastatic assay and the formation of metastatic sites in lungs of nude mice in an spontaneous metastatic assay of human gastric carcinoma cells (Xie et al., 2011). More preclinical studies are required to support the development of clinical trials.

CONCLUSION

Several bioactive molecules from snake venoms have been identified and tested to counteract relevant features of metastatic cancer cells. Further studies are needed to ensure the development of clinical trials and add these molecules to the repertoire of pharmacological treatments against the malignant progression of cancer.

In this mini-review, we focus on collecting data on toxins and compounds isolated from snake venom and their application in metastasis. The continuation of research in this area is urgent, in this sense, novel approaches for the identification of bioactive molecules from snake venom must be developed, and the implementation of virtual methods can accelerate the process through analytical chemistry tools. With thousands of species of snakes on the planet, there is a great diversity of sources to obtain medicine.

The prospect of drug discovery based on natural sources such as herbs, animals and other organisms is growing. Ongoing efforts involve using advances in science and technology to develop new and improved processes that could ultimately lead to new and effective drugs for cancer treatment.

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