Biomedicine & Pharmacotherapy 77 (2016) 14–19

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Review

Tick salivary gland as potential natural source for the discovery of promising antitumor drug candidates

Ana Marisa Chudzinski-Tavassi[a,*], Katia L.P. Morais[a,b], Mário Thiego Fernandes Pacheco[a], Kerly Fernanda Mesquita Pasqualoto[a], Jean Gabriel de Souza[a,b]

[a] Biochemistry and Biophysics Laboratory, Butantan Institute, SP, Brazil
[b] Department of Biochemistry, Federal University of São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 12 August 2015
Accepted 3 November 2015

Keywords:
Amblyomin-X
TFPI-like
Proteasome inhibitor
Autophagy inhibition
Antitumor drug candidate
Anticoagulant

ABSTRACT

Nowadays, the relationship between cancer blood coagulation is well established. Regarding biodiversity and bioprospection, the tick biology has become quite attractive natural source for coagulation inhibitors, since its saliva has a very rich variety of bioactive molecules. For instance, a Kunitz-type FXa inhibitor, named Amblyomin-X, was found through transcriptome of the salivary gland of the *Amblyomma cajennense.* tick. This TFPI-like inhibitor, after obtained as recombinant protein, has presented anticoagulant, antigionenic, and antitumor properties. Although its effects on blood coagulation could be relevant for antitumor effect, Amblyomin-X acts by non-hemostatic mechanisms, such as proteasome inhibition and autophagy inhibition. Notably, cytotoxicity was not observed on non-tumor cells treated with this protein, suggesting some selectivity for tumor cells. Considering the current efforts in order to develop effective anticancer therapies, the findings presented in this review strongly suggest Amblyomin-X as a promising novel antitumor drug candidate.

Contents

1. Cancer, coagulation and ticks—an unexpected relationship? . . . 14
2. Primary sequence comparison and structural features of Amblyomin-X . . . 15
3. Antitumor activity of Amblyomin-X . . . 15
4. Proposed mechanism of action for Amblyomin-X . . . 16
5. Conclusion . . . 18
Conflict-of-interest disclosure . . . 18
Acknowledgements . . . 18
References . . . 18

1. Cancer, coagulation and ticks—an unexpected relationship?

The first hypothesis about a mutual relationship regarding cancer and blood coagulation arose more than a century ago [1,2]. Nowadays, the pathophysiology of cancer-associated thrombosis remains not entirely understood [3,4], but the production of the prime physiological initiator of coagulation, tissue factor (TF), by tumor cells seems to have a role in that scenario [5,6]. In addition, activation of the coagulation cascade appears to support processes of tumor growth, metastasis, and angiogenesis [7,8]. In fact, the antitumor effects of coagulation inhibitors have been described in the last years, including essential hemostatic or non-hemostatic mechanisms [9–11].

The tick biology has been correlated to this theme, since several studies have shown that the saliva is a rich source of bioactive molecules, such as coagulation inhibitors [12–14]. Among these inhibitors, some are structurally similar to the endogenous tissue factor pathway inhibitor (TFPI), which is the physiological FXa

Abbreviations: TF, tissue factor; TFPI, tissue factor pathway inhibitor; EP, electrostatic potential; mTOR, mammalian target of rapamycin; ER stress, reticulum endoplasmic stress; ROS, reactive oxygen species.

* Corresponding author at: Biochemistry and Biophysics Laboratory, Butantan Institute, Av. Vital Brazil 1500, CEP 05503-900, São Paulo, SP, Brazil.
Fax: +55 1126279505.
E-mail address: ana.chudzinski@butantan.gov.br (A.M. Chudzinski-Tavassi).

http://dx.doi.org/10.1016/j.biopha.2015.11.003
0753-3322/

inhibitor, and also has interesting antitumor potential [15,16]. Moreover, recent reports have demonstrated that the tick saliva presents either cytotoxicity to tumor cells, or affect their migratory and invasive activity [13,17].

In this regard, the transcriptome of the salivary gland of the *Amblyomma cajennense* tick was characterized and analyzed by expressed sequence tags (EST) [14]. The study revealed the presence of transcripts related to proteins involved in the hemostatic processes, especially proteases and inhibitors. Among the proteins found was a Kunitz-type inhibitor similar to the tissue factor pathway inhibitor precursor (TFPI) [14], named Amblyomin-X (*Amblyom ma cajennense* in hibitor of factor X a). Later on, this inhibitor was obtained as a recombinant protein (GenBank code AAT68575), and presented anticoagulant, antigionenic, and antitumor proprieties. The structural features and antitumor effect of Amblyomin-X will be exploited in the next topics [18–21].

2. Primary sequence comparison and structural features of Amblyomin-X

Amblyomin-X can be considered functionally related to the TFPI-like inhibitors, since it presents a Kunitz-like domain and experimentally inhibits the extrinsic tenase complex as well as the amidolytic activity of FXa [22,23]. There are conservation patterns in the primary sequence regarding the amino acid residues of the Amblyomin-X Kunitz-type with the second Kunitz domain (KD2) of human TFPI-1 [24] and Kunitz domain 1 (KD1) of human TFPI-2 [25] as well as with another inhibitor from the *Ixodes capsularis* tick, named ixolaris (GI: 15077002, GB: AAK83022.1|AF286029_1). Considering particularly the portion from Cys28 to Cys79 of Amblyomin-X Kunitz-type region, this inhibitor presents 33%, 35%, and 35% of sequence identity with ixolaris, TFPI-1 KD2, and TFPI-2 KD1, respectively. The six cysteine residues in TFPI-1 KD2 and TFPI-2 KD1 are conserved in the Amblyomin-X Kunitz-type domain [22,23].

The three-dimensional (3D) molecular modeling of Amblyomin-X was obtained by homology modeling after 30 ns molecular dynamics simulation [23]. The stability of the conformational arrangement can be attribute to the presence of three disulfide bridges (Cys28-Cys79, Cys39-Cys62, and Cys54-Cys75), which confer more rigidity to the protein structure (Fig. 1A). The backbones of the available 3D molecular models were superimposed based, primarily, upon the Kunitz-type region to reveal common 3D structural features among TFPI-1 KD2 [PDB ID 1ADZ:A [24]], TFPI-2 KD1 [PDB ID 1ZR0:D;[25]], and Amblyomin-X homology model [23]. The Kunitz-type domains from Amblyomin-X (gray) and human TFPIs, TFPI-1 KD2 (pink) and TFPI-2 KD1 (blue), have displayed a very high structural conservation (root mean square deviation, RMSD, values ranging from 0.8 to 0.5 Å; Fig. 1B). Moreover, the electrostatic contributions, which are also important in specific protein–protein recognition, have also been explored [23]. The molecular surfaces of Amblyomin-X and human TFPIs, colored by electrostatic potential (EP), can be seen in Fig. 1C. Only charged residues are taken into account and the interpretation is related to a color scheme (negatively charged surfaces are showed in red, non-charged in white and positively charged in blue). TFPI-1 KD2 and TFPI-2 KD1 showed a quite similar EP on the Kunitz-type region consisting of more negatively charged residues at the upper side and more positively charged at the bottom. However, this pattern was not quite followed by Amblyomin-X, which clearly revealed a more negatively charged profile on the center and a more positively profile on the bottom and upper side of the Kunitz-type domain, evidencing that it would have likely a distinct recognition pattern, at molecular level.

3. Antitumor activity of Amblyomin-X

Although *in vitro* studies have identified the action of Amblyomin-X regarding human pancreas tumor, human melanoma, renal cells carcinoma murine and melanoma murine (next topic of this review), the *in vivo* studies have reported only melanoma murine findings [19,26,27]. For melanoma model, using B16F10 cells line, the Amblyomin-X treatment (1 mg/kg daly, during 14 days) promoted tumor regression regarding the tumor mass and reduction of the number and size of metastasis (Fig. 2) [19]. A comparative study between Amblyomin-X and heparin, in

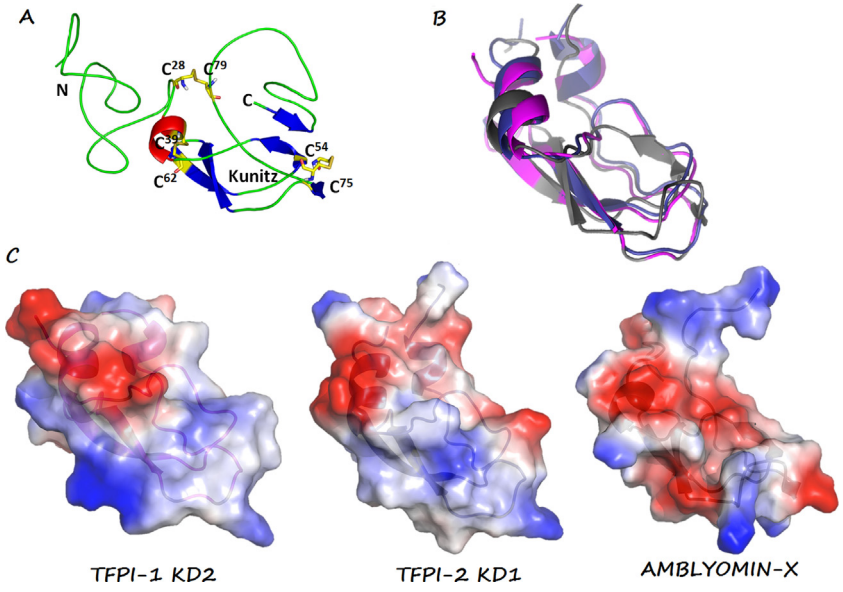

Fig. 1. Structural features of Amblyomin-X. (A) 3D molecular model obtained by comparative homology, after 30 ns simulation: the three disulfide bridges (Cys28-Cys79, Cys39-Cys62, and Cys54-Cys75) confer more rigidity/stability to the protein structure. (B) Backbone superimposition of human TFPIs [24,25] and Amblyomin-X homology model [23] based upon the Kunitz-type region (TFPI-1 KD2 in pink, TFPI-2 KD1 in dark blue, Amblyomin-X in gray); α-helices are shown as spirals, β-sheets as flat arrows, loops as cylinder tubes. (C) Molecular surfaces of human TFPIs (TFPI-1 KD2,[24]; TFPI-2 KD1, [25]) and Amblyomin-X [23], colored by electrostatic potential (EP). The interpretation is related to a color scheme where negatively charged surfaces are showed in red, non-charged in white and positively charged in blue.