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Acta Medica Marisiensis 2016;62(1):106-111 DOI: 10.1515/amma-2015-0114

UPDATE

Snake Venom Metalloproteinases

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As more data are generated from proteome and transcriptome analysis revealing that metalloproteinases represent most of the Viperid and Colubrid venom components authors decided to describe in a short review a classification and some of the multiple activities of snake venom metalloproteinases. SVMPs are classified in three major classes (P-I, P-II and P-III classes) based on the presence of various domain structures and according to their domain organization. Furthermore, P-II and P-III classes were separated in subclasses based on distinctive post-translational modifications. SVMPs are synthesized in a latent form, being activated through a Cys-switch mechanism similar to matrix metalloproteinases. Most of the metalloproteinases of the snake venom are responsible for the hemorrhagic events but also have fibrinogenolytic activity, poses apoptotic activity, activate blood coagulation factor II and X, inhibit platelet aggregation, demonstrating that SVMPs have multiple functions in addition to well-known hemorrhagic function.

Keywords: snake venom metalloproteinases, hemorrhagic activity, hemostatic distruption, ADAM and ADAMTS, SVMPs

Received: 18 August 2015 / Accepted: 21 September 2015

Introduction

Snakes have fascinated humankind for millennia, being worshiped in antiquity by almost all the civilizations. In Ancient Egypt a Cobra adorned the pharaoh's crown, in Greece snakes could be found in many medical symbols, while in India snakes even had their own festival (Nag Panchami) [1,2].The destructive effect of snake venom on living organism was well known, but their healing potential has only been considered in the last 2 centuries. Snake venoms are typically a complex cocktail of mostly peptides, proteins, enzymes and other small substances with toxic and lethal properties that facilitate the immobilization and digestion of the prey, as well as providing a defense against predators. The life or death of these prey/predator encounters forced the toxins to be fast-acting and potent molecules. The most common snake venom enzymes include; cysteine-rich secretory proteins (CRiSPs), three-finger toxins (3FTXs) especially in Elapidae family, phospholipase A_2, metalloproteinases, L-amino acid oxidase, serine proteinases, acetylcholinesterase, and phosphodiesterase [3].

History

Early studies by Reichert and Mitchell noticed that fibrinogen in animal blood loses its ability to coagulate after exposure to snake venom. They observed that when animal blood was mixed with *Crotalus* family venom, the blood clot that was formed was dissolved within 24 hours while sitting at room temperature [4,5]. Probably the first report of SVMPs was by Ohsaka, Okonogi and Maeno when they observed that isolated proteinases toxin from viperid were inactivated by exposure to EDTA, presumably because of the presence of a metal inside the protein, most likely being a metalloproteinase [6-9].

Since the discovery of zinc-dependent proteinase in the viperid venom [10], researchers have tried to isolate, characterize and understand the structure and biological function in order to find their role in the envenomation of the prey [11]. Proteinase represents one of the enzymes found in the venom of many snakes, and are structurally classified as serine proteinases and metalloproteinases [12]. The most intriguing enzyme is metalloproteinase and it is estimated that it is at least 30% of the total protein content of most viperid venoms [13]. Soon after the discovery of the snake venom metalloproteinases and the first sequence determination of them, it was thought that they are part of the matrix metalloproteinases family [14]. However, Bjarnason and Fox discovered that snake venom metalloproteinases were part of a new family of metalloproteinases, distinct from matrix metalloproteinase [15,16].

Classification

As major classes, proteinase can be classified in exopeptidase (*e.g.* aminopeptidase, cathepsin A) and endopeptidase (ADAMs and matrix metalloproteianse). ADAMs stands for ***A D***isintegrin ***A***nd ***M***etalloproteinase, while ADAMTs are ***A D***isintegrin ***A***nd ***M***etalloproteinase with ***T***hrombospondin motifs.

SVMPs were originally classified into structural classes based on the presence of various domain structures and according to their domain organization [15]. All SVMPs share a metalloproteinase domain where the metallic ion is either zinc (most of the metalloproteinases from the snake venom) or cobalt, characterized by the HEXXHXXGXXH metal (zinc)-binding motif. Being an extracellular protein, it is expected to have structurally stabilizing disulfide bonds. cDNA sequence analysis indicate that SVMPs are synthesized in a latent form, being

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activated through a Cysteine-switch mechanism similar to matrix metalloproteinases [17,18].

Schematically, snake venom metalloproteinases can be classified in three major classes (figure 1), originally developed by Fox and Serrano in 2005 [19]. Usually ADAMs are multi-domain proteins that are minimally comprised of metalloproteinase and disintegrin-like domains. In addition, a peptide signaling sequence can be found in the beginning of the protein chain. Some ADAMs also display additional domains, such as transmembrane and cytoplasmic, as well as thrombospondin domains [20].

The first class, named P-I is the simplest SVMPs, comprised of only a metalloproteinase domain. In their nascent form, they have a peptide signal sequence, a pre-pro-domain, which undergoproteolytically in the mature SVMPs, followed by a "*spacer*" domain towards with carboxy group to metalloproteinase domain. The molecular weight of these classes of metalloproteinaseslies around 25 kDa, with 2 sub-domains, a major one (4 α–helix and β-folding) and a minor (with only one α–helix). Usually P-I class has 4 to 6 cysteine residues in the protein.

The second class of SVMPs is P-II, which is somewhat similar to P-I class, being characterized by the presence of a disintegrin domain in the nascent form, which in most cases hosts the canonical RGD (arginine-glycine-aspartic acid) motif. The P-II class was subdivided in 5 different subclasses (P-IIa, P-IIb, P-IIc, P-IId and P-IIe) of metalloproteinases. The difference between all subclasses arise on the disintegrin domain based on their distinctive post-translational modifications.

The disintegrin domain is found carboxy to the spacer domain, which can be modified proteolytically to a "free" disintegrin. The molecular weight of P-II classes is between 25 and 50 kDa.

In P-IIb disintegrin domain is not processed, remaining part of the proteinase, while P-IIc is in fact a dimeric form of P-IIb. P-IId is a precursor from which a homodimeric disintegrin found in venoms is formed, while P-IIe represents the actual P-II class of snake venom metalloproteinases, which give rise to a heterodimeric RGD-containing disintegrin [21].

The P-III SVMPs family has much more domains than P-II class, bearing signaling peptide sequence, pro-domain, metalloproteinase, spacer, disintegrin-like and cysteine-rich domains (figure 2) in the nascent forms, carboxy to the spacer domain. P-III family is divided in 4 subclasses having differences along the disintegrin-like and cysteine-rich domains.

It is one of the heaviest family of metalloproteinases often having molecular weight of more than 50 kDa. Even

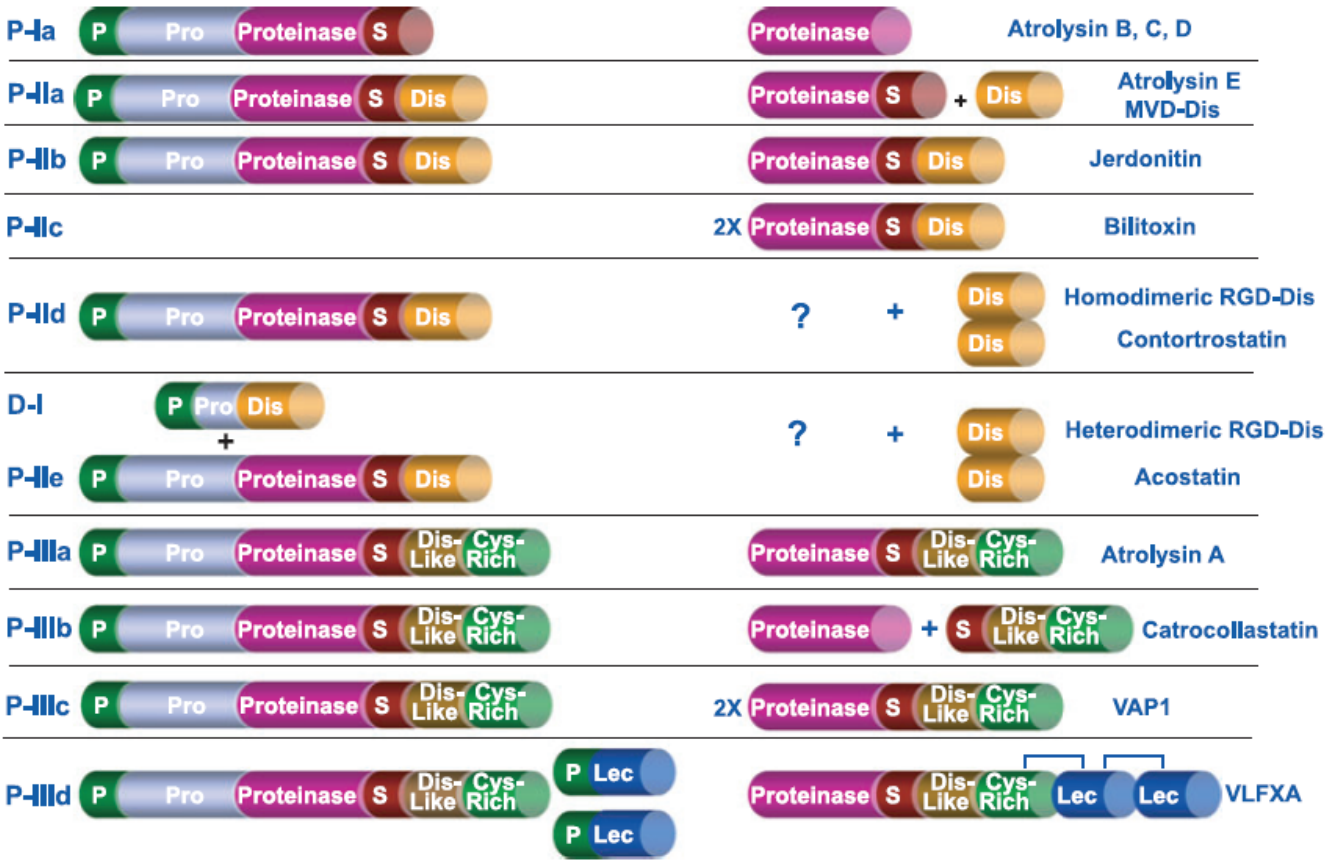

Fig. 1. Schematic of snake venom metalloproteinases classes (reprinted from Fox and Serrano, 2008 [13], with permission from Wiley). Question marks (?) from the figure indicate the lack of processed product identification in the venom. In the figure, P means signaling peptide sequence, Pro means a pro-domain, S stands for "spacer", Dis is disintegrin or Dis-Like a disintegrin-like domain, Cys-Rich represents a cysteine-rich domain and Lec is lectin domain.

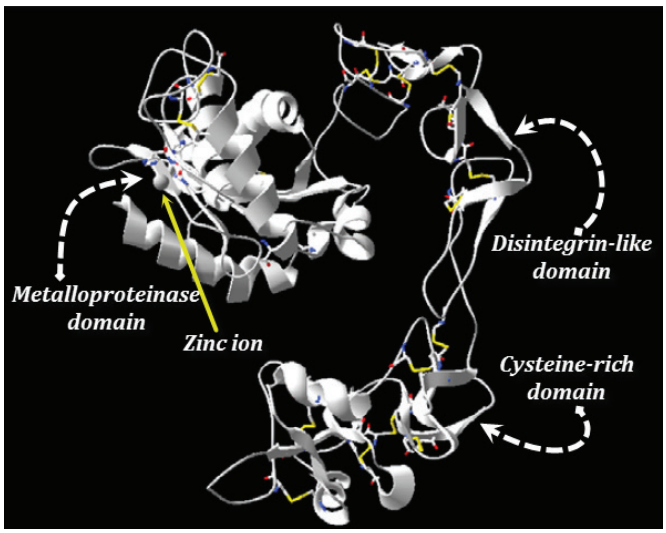

Fig. 2. Structural analysis of the P-III SVMP jararhagin model (reprinted from Pinto and Fox, 2007 [22], from Elsevier)

if disintegrin-like domain shares some sequence homology with the disintegrin domain of the P-II family, they are distinct, having different disulfide bond structure.

P-IIIa is somewhat similar to P-IIb, where disintegrin-like and cysteine-rich domains do not undergo any post-translational modifications. In contrast, in the P-IIIb subclass, the spacer, the disintegrin-like and cysteine-rich domains are proteolytically modified from the metalloproteinase domain [21]. Up to date, there is uncertainty about the processing mechanism for the metalloproteinase domain of the spacer, disintegrin-like and cysteine-rich domains. Moura and coworkers, however, suggested that the processing of the domains from the proteinase is linked with the disulfide bond arrangement and/or folding of the protein [23].

P-IIIc is practically the dimeric form of P-IIIa, while P-IIId subclass is a modified P-IIIa structure with the addition of two disulfide-bonded lectin-like domains post-translationally coupled to the P-IIIa structure.

Proteomic analysis of snake venom for metalloproteinases and SVMPs structure

Recent proteomic analyses of snake venom have shown that SVMPs are the major components of venom of Viperidae family, varying from roughly 11% up to almost 65% [24]. But SVMPs are ubiquitously distributed in almost all venomous snake species being present in Colubridae family [25,26] as well as in the Elapidae (in some members) family [27,28] although in much lower quantity than in Viperidae family.

Practically almost all of the SVMPs are zinc-dependent but cobalt can be also involved. The metal inside the metalloproteinase is tetra coordinated, three ligands being coordinated from protein (usually histidine, lysine, arginine etc.) and one with a labile water molecule.

Inactivation of snake venom metalloproteinases can be done using chelating agent such as ethylenediaminetetraacetic acid (EDTA) or o-phenanthroline [21].

SVMPs are synthetized in cytoplasm of specialized secretory glands cells and transferred to the rough endoplasmic reticulum, then to the Golgi apparatus, and finally transported to the lumen of the venom gland viasecretory granules.

Studying cDNA Fox and Serrano observed that every metalloproteinases from the snake venom have a signaling sequence that probably targets a nascent protein to a signal recognition site or protein on the endoplasmic reticulum. The peptide signal sequence removing occur during transport into to endoplasmic reticulum (ER). It has also been presumed that folding of the protein takes place in the rough ER and undergo disulfide bond oxidation/formation, glycosylation or dimerization/polymerization of the nascent proteins [13].

Incorrectly, folded proteins, which takes place in the venom gland secretory, will not be transferred to the Golgi network. Fox suggested that in the trans-Golgi network nascent preform of the proteins would be proteolytically processed [13], as it happens with most of the latent proteins. The process is most probably completed at the time that the vesicles are released into the gland lumen (figure 3).

Shimokawa and collaborators have analyzed a recombinant pro-atrolysin E (a P-IIa SVMP from *Crotalus atrox*) and they have noticed, "*in vitro*" an autolytic processing which occur via an initial proteolytic cleavage to release the pro-domain followed by a second cleavage to the disintegrin domain [18]. They also noticed that SVMPs have a functional Cysteine-switch mechanism for maintaining the protein in a latent form. Analyzing a P-IIIb class SVMP jararhagin from *Bothrops jararaca* Shimokawa [28] and Moura-da-Silva [23] observed that this class undergoes to a post-translation process where a disintegrin-like/cysteine-rich domain are released giving rise to a jararhagin-C, but not on entire population of the proteins. There are still part of the proteins population that do not undergo on this post-processing, one possible explanations could be that there are different folding isomers and they can be differentially processed in order to release disintegrin-like/cysteine-rich domain, explaining the snake venom complexity and function.

Another important characteristic of SVMPs structure are disulfide bonds. Usually a P-I subclass has two to three disulfide bonds in the metalloproteinase domain. In the P-II and P-III subclasses that have often an odd number of cysteinyl residues there are slightly more disulfide-bonds inside the metalloproteinase domain. In the P-II and P-III subclasses cysteinyl residue can be found as free cysteine or form disulfide bonds not only inside a specific domain but also with cysteinyl residues from other domains, or forming dimeric or polymeric structures.

P-II subclass usually exhibit about 5 to 7 disulfide bonds inside the disintegrin domains, while P-III subclass has around 8 disulfide bonds in the disintegrin-like domain, 6 in the cysteine-rich domain and 3 disulfide bonds in the lectin-like domain of P-IIId.

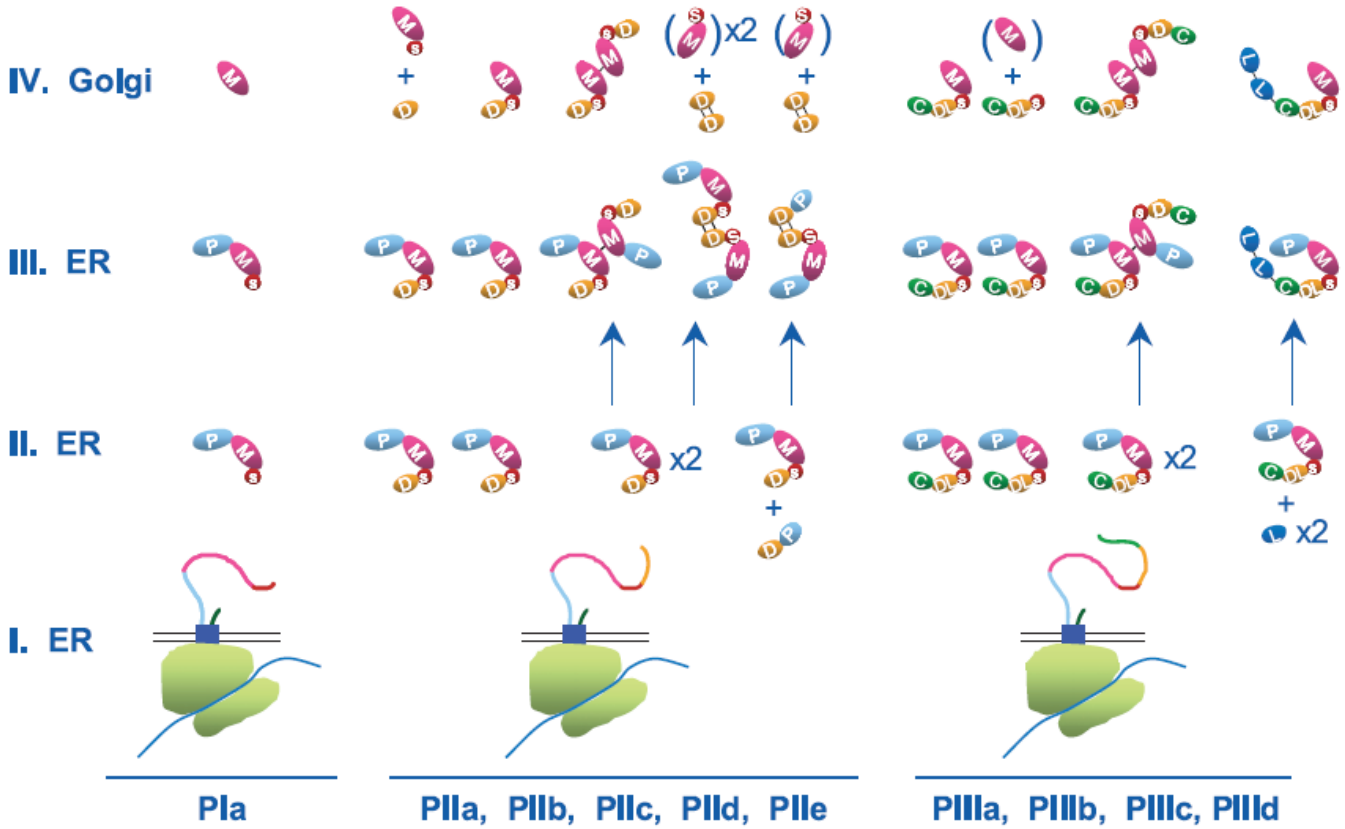

Fig. 3. Schematic representing a hypothetical biosynthesis pathway from transcription on the ER surface, through endoplasmic reticulum to the Golgi network and release of the secretory vesicles into the venom lumen for the production of the three SVMPs venom classes. In the figure, P means a pro-domain, M is metalloproteinase domain, S stands for "spacer", D is disintegrin or DL a disintegrin-like domain, Cys represents a cysteine-rich domain and L is lectin-like domain (reprinted from Fox and Serrano, 2008 [13], with permission from Wiley).

Function of SVMPs

Wide varieties of biochemical and biological activities have been described to the SVMP, showing therefore their critical role in the overall toxicity of the venom. SVMPs are responsible for the local and systemic hemorrhagic activity, myonecrosis [17], disruption of hemostasis mediated by procoagulant or anticoagulant effects, platelet aggregation, necrosis or pro-inflamatory activities. Therefore, the most important role of metalloproteinases seems to be in the prey predigestion. Table I summarize some of the activities of some metalloproteinases isolated from snake venom.

Table I exhibits a brief summary of some of the biological activities associated with some SVMPs. As it can be seen most of the functional activities of SVMPs are associated with hemorrhagic or the disruption of the hemostatic system. All these activities are mediated by the proteolytic activity of metalloproteinase domain of SNVMs. Blood coagulation proteins (*e.g.* fibrinogen, factor X and factor II, prothrombin) are also targets of their proteolytic activities showing fibrinogenolytic activity, inhibition of platelet aggregation and activation of factor II and X.

Conclusions

Since SVMPs represent an important and major class of snake venom proteins as revealed by proteomic analysis and many of these proteinase have biological activity we have collected new data, especially after 2000, in a mini-review. In this mini-review, we intended to present, based on literature data, the current understanding of the structural features that contribute to complexity of snake venom metalloproteinases. There is described classification of SVMPs as well as biological function of them. Most of the metalloproteinases of the snake venom are responsible for the hemorrhagic events that occur in the case of envenomation therefore represents a significant health hazard for the victims. We believed the venoms of snake could represent a huge and important source for potent pharmacological active molecules or for lead molecules that should be explores as much as possible.

Acknowledgments

This paper was published under the frame of European Social Found, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/136893.

We would like to thanks to Michael Holmes (Canada) for a brief review, drafting and editing the article in English.

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Table I. Biological activities of selected SVMPs

SVMP Class	Isolated SVMP	Sources	Activity/Activities	References
P-I class	Atrolysisn C	Crotalus atrox	Hemorrhagic	Kress&Paroski [30]
	Adamalysin	Crotalus adamanteus	Inhibition of serum proteinase inhibitors	Zhang et al. [31]
	BaP1	*Bothrops asper*	Hemorrhagic, myonecrotic, inflammatory	Gutierrez et al. [32] Rucavado et al. [33]
	H2-proteinase	*Trimeresurus flavoviridis*	Proteolytic, non-hemorrhagic	Takeya et al. [34]
	Graminelysin I	*Trimeresurus gramineus*	Apoptotic	Wu et al. [35]
	BmooMPα-I	*Bothrops moojeni*	Fibrin(ogen)olytic	Bernardes et al. [36]
	Batroxase	*Bothrops atrox*	Fibrin(ogen)olytic and thrombolytic	Cintra et al. [37] de Toniet al. [38]
	BpMP-II	*Bothrops pauloensis*	Fibrin(ogen)olytic, non-hemorrhagic on intramuscular administration and inhibit angiogenesis	Achê et al. [39]
P-II class	Jerdonitin	*Trimeresurus jedonii I*	Inhibition of platelet aggregation	Chen et al. [40]
	Bilitoxin-1	*Agkistrodon bilineatus*	Hemorrhagic	Nikai et al. [41]
	MD-d	*Agkistrodon halys brevicaudus*	Proteolytic	Jeon&Kim [42]
	BlatH1	*Bothriechis lateralis*	Fibrin(ogen)olytic, Inhibition of platelet aggregation, Hemorrhagic	Camacho et al. [43]
P-III class	R-Ele-1	*Protobothrops elegans*	Fibrin(ogen)olytic	Oyama&Takahashi [44]
	SV-PAD-2	*Protobothrops elegans*	Inhibition of platelet aggregation Fibrin(ogen)olytic	Oyama&Takahashi [44]
	VaF1	*Viper ammodytes ammodytes*	Fibrin(ogen)olytic and Non-hemorrhagic via i.m.	Leonardi et al. [45]
	Flavorase (SSP-1 up to SSP-5)	*Protobothrops flavoviridis*	Non-hemorrhagic and Non-cytotoxic	Shioi et al. [46]
	Jarahagin	*Bothrops jararaha*	Inhibition of platelet aggregation, Hemorrhagic	Paine [47]
	Moojenactivase	*Bothrops moojeni*	Activation of factor II and X	Sartim et al. [48]

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