biology and life sciences forum

MDPI

Proceedings

Newly identified toxin transcripts in Myanmar Russell's viper venom gland

Khin Than Yee [1,*], Jason Macrander [2], Olga Vasieva [3,4] and Ponlapat Rojnuckarin [5]

[1] Department of Medical Research, Yangon, Myanmar 1; khinthanyee@gmail.com
[2] Florida Southern College, Lakeland, USA 2; jmacrander@flsouthern.edu
[3] Biosynthetic Machines Inc (BSMI), USA 3; ovasieva@ingenets.com
[4] Institute of Integrative Biology, University of Liverpool, Liverpool, UK
[5] Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand 5; rojnuckarinp@gmail.com
* Correspondence: khinthanyee@gmail.com; Tel.: +95943023542.

Abstract: Russell's viper (*Daboia siamensis*) is a medically important snake in Myanmar due to its high morbidity and mortality. The genome of Myanmar Russell's viper has not been sequenced until recently. Hence, RNA-Sequencing has been used to predict genes encoding this snake's toxins. This can lead to deeper insights in pathogenesis of envenoming and potential drug discovery. Venom glands were dissected from four adult *D. siamensis* specimens (two males and two females) provided by a local Myanmar Snake Farm. The mRNA was extracted and sequenced on the Illumina HiSeq platform, then assembled *de novo* using the Trinity software. Candidate toxin genes were identified using the Venomix pipeline and their expression levels were calculated by mean of RSEM software. Identified toxin candidates were aligned with previously described venom proteins using Clustal Omega. Candidate venom transcripts were classified into 23 toxin gene families, which included 53 unique transcripts identified as full-length sequences. Among them, 28 full-length sequences represented the eight newly identified toxin gene families in *D. siamensis* including Neprilysin (2), Cystatin (5), Waprin (1), Vipericidin (1), Veficolin (1), Endothelial lipases (9), Vespryn (ohanin) (8) and three-finger toxins (1). Their expression levels were found to be moderate to low (TPM= 1.49 to 213.37). The majority of the toxin candidates resembled typical elapid toxins, which usually exhibit neurotoxic activities and tissue damage. A smaller proportion of candidate toxin transcripts were predicted to display antimicrobial activity and anti-metastatic effect. Our results suggest their functional activities. They should be studied further for potential therapeutic applications.

Keywords: Russell's viper 1; venom gland 2; toxin transcript 3

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Published: date

1. Introduction

Russell's viper (*Daboia siamensis*) is a medically important snake in Myanmar due to its high morbidity and mortality. The genome of Myanmar Russell's viper has not been sequenced until recently. Hence, RNA-Sequencing has been used to predict genes encoding this snake's toxins. This can lead to deeper insights in pathogenesis of envenoming and potential drug discovery [1].

2. Methods

Venom glands were dissected from four adult *D. siamensis* specimens (two males and two females) provided by a local Myanmar Snake Farm. The mRNA was extracted and sequenced on the Illumina HiSeq platform, then assembled *de novo* using the Trinity software. Candidate toxin genes were identified using the Venomix pipeline and their

expression levels were calculated by mean of RSEM software. Identified toxin candidates were aligned with previously described venom proteins using Clustal Omega.

3. Results and Discussion

Candidate venom transcripts were classified into 23 toxin gene families, which included 53 unique transcripts identified as full-length sequences. Among them, 28 full-length sequences represented the eight newly identified toxin gene families in *D. siamensis*: Neprilysin (2), Cystatin (5), Waprin (1), Vipericidin (1), Veficolin (1), Endothelial lipases (9), Vespryn (ohanin) (8) and three-finger toxins (1). Their expression levels were found to be moderate to low (TPM= 1.49 to 213.37). The majority of the toxin candidates were resembled to typical elapid toxins, which usually exhibit neurotoxic activities and tissue damage. A smaller proportion of candidate toxin transcripts were predicted to display antimicrobial activity and anti-metastatic effect (Table 1).

Table 1. Rarely and newly found toxin genes in Myanmar Russell's viper transcriptome.

No.	Toxin family	Function	No. of full-length	TPM	Snakes species from NCBI hit	Notes (originally the toxin isolated)
1.	Neprilysin	Inactivation of peptide transmitters at synapses	2	64.38-213.37	*Vipera anatolica senliki* (Viperidae)	Their presence in snake venoms (*Ophiophagus Hannah*, *Echis pyramidum leakeyi*, *Naja kaouthia*, and *Crotalus horridus*), scopion, gellyfish and hunting wasps (insect). [2]
2.	Cystatin	Cysteine protease inhibitors and Anti-metastatic effect	5	8.99-113.67	*Crotalus adamanteu* (Viperidae), *Protobothrops mucrosquamatus* (Viperidae)	Snake venom cystatin (sv-cystatin) was isolated from snake venom of *Naja naja atra*. [3]
3.	Waprin	Diverse functions and antibacterial activity	1	10.34	*Philodryas olfersii* (Colubridae)	Nawaprin, 1st member of the snake waprin family was purified from the venom of *Naja nigricolis* [4]
4.	Vipericidin	Antimicrobial activity	1	3.13	*Pantherophis guttatus* (Colubridae)	Cathelicidins were found in Chinese cobra (*Naja atra*), King cobra (*Ophiophagus hannah*) & Banded krait (*Bungarus fasciatus*). [5]
5.	Veficolin	Inhibition of platelet aggregation and/or blood coagulation	1	2.77	*Pantherophis guttatus* (Colubridae)	Veficolin was newly identified in *Cerberus rynchops* (dog face

						water snake) (Colubridae).[6]
6.	Endothelial lipases	Allergic reactions	9	1.75-2.84	*Vipera anatolica senliki* (Viperidae)	The major part of venom allergens in wasps (Hymenoptera insects) is phospholipase A1. [7]
7.	Vespryn (Ohanin)	Neurotoxicity	8	2.25-12.14	*Ophiophagus Hannah* (Elapidae)	A novel protein, ohanin from king cobra venom was firstly identified, purified and functionally characterized. [8]
8.	Three-finger toxins	Neurotoxicity and tissue damage	1	1.49	*Lachesis muta* (Viperidae)	3FTs are predominant toxins in Elapidae venoms. α-bungarotoxin from *B. multicinctus* venom blocks the muscle-type (α1)2$\beta\gamma\delta$ nAChR, firstly shown by Chang and Lee (1963). [9]

4. Conclusions

Minor venom proteins from Myanmar Russell's viper were explored at transcript level by transcriptomic approach. Neprilysin, cystatin, waprin, vipericidin, veficolin, endothelial lipases, vespryn and three-finger toxins were newly identified from Myanmar Russell's viper transcriptomes. Our results suggest their functional activities. They should be studied further for potential therapeutic applications.

5. Conflict of interest

The authors declare no conflict of interest.

References

[1] Cañas CA, Castaño-Valencia S, Castro-Herrera F, Cañas F, Tobón GJ. Biomedical applications of snake venom: from basic science to autoimmunity and rheumatology. J Transl Autoimmun 2021;4. https://doi.org/10.1016/j.jtauto.2020.100076.

[2] do Nascimento SM, de Oliveira UC, Nishiyama-Jr MY, Tashima AK, Silva Junior PI da. Presence of a neprilysin on *Avicularia juruensis* (Mygalomorphae: Theraphosidae) venom. Toxin Rev 2022;41:370–9.

https://doi.org/10.1080/15569543.2021.1878226.

[3] Brillard-Bourdet M, Nguyên V, Ferrer-Di Martino M, Gauthier F, Moreau T. Purification and characterization of a new cystatin inhibitor from Taiwan cobra (*Naja naja atra*) venom. Biochem J 1998;331:239–44. https://doi.org/10.1042/bj3310239.

[4] Torres AM, Wong HY, Desai M, Moochhala S, Kuchel PW, Kini RM. Identification of a novel family of proteins in snake venoms. Purification and structural characterization of nawaprin from *Naja nigricollis* snake venom. J Biol Chem 2003;278:40097–104. https://doi.org/10.1074/jbc.M305322200.

[5] Zhao H, Gan T-X, Liu X-D, Jin Y, Lee W-H, Shen J-H, et al. Identification and characterization of novel reptile cathelicidins from elapid snakes. Peptides 2008,29:1685-1691.

[6] Ompraba G, Chapeaurouge A, Doley R, Devi KR, Padmanaban P, Venkatraman C, et al. Identification of a novel family of snake venom proteins veficolins from *Cerberus rynchops* using a venom gland transcriptomics and proteomics approach. J Proteome Res 2010;9:1882–93. https://doi.org/10.1021/pr901044x.

[7] King TP, Kochoumian L, Joslyn A. Wasp venom proteins: Phospholipase A1 and B. Arch Biochem Biophys 1984;230:1–12. https://doi.org/https://doi.org/10.1016/0003-9861(84)90080-8.

[8] Yuh FP, Wong PTH, Kumar PP, Hodgson WC, Kini RM. Ohanin, a novel protein from king cobra venom, induces hypolocomotion and hyperalgesia in mice. J Biol Chem 2005;280:13137–47. https://doi.org/10.1074/jbc.M414137200.

[9] Chang CC, Lee CY. Isolation of neurotoxins from the venom of *Bungarus multicinctus* and their modes of neuromuscular blocking action. Arch Int Pharmacodyn Ther 1963;144:241-257.