biology and life sciences forum

MDPI

Proceeding Paper

Newly Identified Toxin Transcripts in Myanmar Russell's Viper Venom Gland †

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

[1] Department of Medical Research, Yangon 11191, Myanmar
[2] Biology Department, Florida Southern College, Lakeland, FL 3301, USA; jmacrander@flsouthern.edu
[3] Institute of Integrative Biology, University of Liverpool, Liverpool L69 3BX, UK; ovasieva@ingenets.com
[4] Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand; rojnuckarinp@gmail.com
* Correspondence: khinthanyee@gmail.com; Tel.: +95-943023542
† Presented at the 2nd International Electronic Conference on Toxins, 14–28 July 2023; Available online: https://iect2023.sciforum.net/.

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 had not been sequenced until recently. Hence, RNA sequencing has been used to predict genes that encode this snake's toxins. This can lead to deeper insights into the 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 using RSEM software. The 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 effects. Our results suggest their functional activities. They should be studied further for potential therapeutic applications.

Keywords: Russell's viper; venom gland; toxin transcript

check for updates

Citation: Yee, K.T.; Macrander, J.; Vasieva, O.; Rojnuckarin, P. Newly Identified Toxin Transcripts in Myanmar Russell's Viper Venom Gland. *Biol. Life Sci. Forum* **2023**, *24*, 11. https://doi.org/10.3390/IECT2023-14796

Academic Editor: R. Manjunatha Kini

Published: 9 October 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

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 had not been sequenced until recently. Hence, RNA sequencing has been used to predict genes that encode this snake's toxins. This can lead to deeper insights into the 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 using RSEM software. The 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 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 effects (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	Snake 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, first 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*) and 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 first 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)2βγδ nAChR, first shown by Chang and Lee (1963). [9]

4. Conclusions

Minor venom proteins from Myanmar Russell's viper were explored at a transcript level using a 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.

Author Contributions: Conceptualization, P.R. and K.T.Y.; methodology, K.T.Y.; software, J.M.; validation, P.R., J.M. and O.V.; formal analysis, K.T.Y. and J.M.; investigation, K.T.Y. and J.M.; resources, P.R.; data curation, J.M. and O.V.; writing—original draft preparation, K.T.Y.; writing—review and editing, P.R., K.T.Y., J.M. and O.V.; visualization, P.R., J.M. and O.V.; supervision, P.R. and O.V.; project administration, P.R.; funding acquisition, P.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Thailand Science Research and Innovation Fund Chulalongkorn University, CU_FRB65_hea(17)_024_30.

Institutional Review Board Statement: This study was approved by the Animal Care and Use Committee, Chulalongkorn University (CU-ACUC) (Protocol code 17/2552) on 28 July 2015.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data of the transcriptome assembly were uploaded to NCBI database under BioProject PRJNA545823, Biosample SAMN11938797 for males and SAMN11939170 for females.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Cañas, C.A.; Castaño-Valencia, S.; Castro-Herrera, F.; Cañas, F.; Tobón, G.J. Biomedical applications of snake venom: From basic science to autoimmunity and rheumatology. *J. Transl. Autoimmun.* **2021**, *4*, 100076. [CrossRef] [PubMed]
2. Do Nascimento, S.M.; de Oliveira, U.C.; Nishiyama, M.Y., Jr.; Tashima, A.K.; da Silva Junior, P.I. Presence of a neprilysin on *Avicularia juruensis* (Mygalomorphae: Theraphosidae) venom. *Toxin. Rev.* **2022**, *41*, 370–379. [CrossRef]
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*, 239–244. [CrossRef] [PubMed]
4. Torres, A.M.; Wong, H.Y.; Desai, M.; Moochhala, S.; Kuchel, P.W.; Kini, R.M. 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*, 40097–40104. [CrossRef] [PubMed]
5. Zhao, H.; Gan, T.-X.; Liu, X.-D.; Jin, Y.; Lee, W.-H.; Shen, J.-H.; Zhang, Y. Identification and characterization of novel reptile cathelicidins from elapid snakes. *Peptides* **2008**, *29*, 1685–1691. [CrossRef] [PubMed]
6. Ompraba, G.; Chapeaurouge, A.; Doley, R.; Devi, K.R.; Padmanaban, P.; Venkatraman, C.; Velmurugan, D.; Lin, Q.; Kini, R.M. 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*, 1882–1893. [CrossRef] [PubMed]
7. King, T.P.; Kochoumian, L.; Joslyn, A. Wasp venom proteins: Phospholipase A1 and B. *Arch. Biochem. Biophys.* **1984**, *230*, 1–12. [CrossRef]
8. Yuh, F.P.; Wong, P.T.H.; Kumar, P.P.; Hodgson, W.C.; Kini, R.M. Ohanin, a novel protein from king cobra venom, induces hypolocomotion and hyperalgesia in mice. *J. Biol. Chem.* **2005**, *280*, 13137–13147. [CrossRef]
9. Chang, C.C.; Lee, C.Y. Isolation of neurotoxins from the venom of *Bungarus multicinctus* and their modes of neuromuscular blocking action. *Arch. Int. Pharmacodyn. Ther.* **1963**, *144*, 241–257. [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.