Discovery of Novel Cell-Penetrating Antimicrobial Peptides by Transcriptome Deep Analysis

Grafskaia E.N.[1,2], Babenko V.V.[2], Anikanov N.A.[2], Polina N.[2]., Kharlampieva D.D.[2], Bobrovsky P.A.[2], Lazarev V.N.[1,2]

[1] *Moscow Institute of Physics and Technology (State University), Dolgoprudny,*
[2]*Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow*
grafskayacath@gmail.com

Cell-penetrating peptides (CPPs) are short cationic peptides able to translocate across the plasma membrane without causing its disruption. That type of peptides is generally used as a cargo carrier for intracellularly delivery of various active molecules, such as drugs. CPPs with antimicrobial efficiency itself may act as antimicrobial agents and kill a wide range of microorganisms[1,2]. Therefore, this work presents the procedure to discover new cell-penetrating peptides active against various pathogens due to the interaction with the intracellular target.

Previously, it was shown the cell-penetrating ability of different peptides contained in the venoms of various predators, such as poisonous snakes, scorpions and spiders[3,4]. Moreover, that peptides exhibit antimicrobial activity[5]. Consequently, the main idea of the research involves the search of toxin-like proteins contains cell-penetrating peptide(s) with antimicrobial potential (CPAMP). The search for CPAMP was performed with the use of the transcriptomic profiling of the tentacles secretion of the sea anemone *Cnidopus japonicus* and the venom glands secretion of the spider *Poecilotheria fasciata*. For the reconstruction of full-length transcripts the software package Trinity was used[6]. Further, identifying of candidate coding regions within transcript sequences were carried out using the software package Transdecoder[7]. Selected transcripts were translated in six frames, and the ORFs encoding the protein more than 10 aa were collected.

Thereafter, the prediction of the presence and location of signal peptides in sorted proteins was fulfilled using the server SignalP[8]. The sequences contained signal peptides were selected. In the following, the mature peptide sequence of toxins was identified according to Processing Quadruplet Motif (PQM): R(K)toR and EtoR/EafterR[9,10]. Additionally, the analysis of CPAMP by their physicochemical properties were accomplished using the R packages "protr"[11], "seqinr"[12], "Peptides"[13]. With the help of applied functions, peptides were withdrawn according to their characteristics correspond to CPAMP. Specifically, the length is less 60 a.o., pI value is in the range of 8 to 12, the positive charge under pH 7.0, the content of proline residues above 30%, the content of tryptophan residues less 25%, the content of arginine or lysine residues less 30%. Within the selected amino acid sequences, the stretches with the highest antimicrobial potential and the ability to penetrate the cell membrane were identified. For this purpose, predictors such as CellPPD[14] (> 0.6), CPPpred[15] (> 0.7), AMPA[16], ADAM[17] (> 1) and CAMP[18] (> 0.5) were used. Applied values of

parameters of each predictor are stated in brackets. Additionally, the collected peptides of the sea anemone were verified against the proteome of *C. japonicus* secretion.

For the moment, we have obtained lists of candidate peptides that likely penetrate the cell membrane without causing any damaging and exhibit antimicrobial activity against intracellular targets. Hereafter, candidate CPAMPs will be chemically synthesised using the Fmoc-based solid-phase strategy. The antimicrobial activity of selected peptides will be determined against *Bacillus subtilis*, *Escherichia coli* by broth microdilution method[19] and against intracellular pathogen *Chlamydia trachomatis*.

This work was supported by research project № 16-34-00936 mol_a from the Russian Foundation for Basic Research (RFBR).

1. Splith, K. & Neundorf, I. Antimicrobial peptides with cell-penetrating peptide properties and vice versa. *Eur. Biophys. J.* **40,** 387–397 (2011).
2. Scocchi, M. *et al*. Proline-rich antimicrobial peptides: Converging to a non-lytic mechanism of action. *Cell. Mol. Life Sci.* **68,** 2317–2330 (2011).
3. Milletti, F. Cell-penetrating peptides: Classes, origin, and current landscape. *Drug Discov. Today* **17,** 850–860 (2012).
4. Ponnappan, N. & Chugh, A. Cell-penetrating and cargo-delivery ability of a spider toxin-derived peptide in mammalian cells. *Eur. J. Pharm. Biopharm.* **114,** 145–153 (2017).
5. Kerkis, I. *et al.* State of the art in the studies on crotamine, a cell penetrating peptide from South American rattlesnake. *Biomed Res. Int.* **2014,** (2014).
6. Grabherr, M. G. *et al.* Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nat. Biotechnol.* **29,** 644–652 (2013).
7. Haas, Brian J. *et al*, manuscript in prep. http://transdecoder.github.io)
8. Petersen, T. N. *et al*. SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nat. Methods* **8,** 785–6 (2011).
9. Kozlov, S. *et al.* A novel strategy for the identification of toxinlike structures in spider venom. *Proteins Struct. Funct. Genet.* **59,** 131–140 (2005).
10. Kozlov, S. A. & Grishin, E. V. The universal algorithm of maturation for secretory and excretory protein precursors. *Toxicon* **49,** 721–726 (2007).
11. Xiao, N. *et al*. protr : R package for generating various numerical representation schemes of protein sequence. (2014).
12. Charif, D. *et al.* Package ‘seqinr’. (2016).
13. Indices, T. C. *et al*. Package ‘Peptides’. (2015).
14. Gautam, A. *et al.* In silico approaches for designing highly effective cell penetrating peptides.

J. Transl. Med. **11,** 1 (2013).

15. Holton, T. A. *et al*. CPPpred: Prediction of cell penetrating peptides. *Bioinformatics* **29,** 3094–3096 (2013).

16. Torrent, M. *et al.* AMPA: An automated web server for prediction of protein antimicrobial regions. *Bioinformatics* **28,** 130–131 (2012).

17. Lee, H.-T. *et al*. A Large-Scale Structural Classification of Antimicrobial Peptides. *Biomed Res. Int.* **2015,** 475062 (2015).

18. Waghu, F. H. *et al*. CAMP R3 : a database on sequences, structures and signatures of antimicrobial peptides. *Nucleic Acids Res.* **44,** gkv1051 (2015).

19. Wiegand, I. *et al*. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat. Protoc.* **3,** 163–175 (2008).