http://dx.doi.org/10.7124/bc.000AE8

Predicting proteases integrated with membranes of gram-negative bacteria andtheir putative roles

N.O. Kozyrovska[1], O.V. Podolich[1], I.Ye. Zaets[1], O.A. Havryliuk[2], H.B. Melnyk[1], L.A. Zaika[1], G.V. Zubova[1]

[1] Institute of Molecular Biology and Genetics, NAS of Ukraine
150, Akademika Zabolotnoho Str., Kyiv, Ukraine, 03143
[2] D.K. Zabolotny Institute of Microbiology and Virology, NAS of Ukraine
154, Akademika Zabolotnoho Str., Kyiv, Ukraine, 03143
kozyrna@ukr.net

Background/Aim. Bacteria produce extracellular membrane vesicles (EV), which now are being investigated for their application as immunotherapeutics, vaccines, and drug delivery vehicles with promising early results [1–3]. The properties of EVs are harnessed for the development of next-generation microbe-inspired therapies. The immunologic effects as the main mechanism of EV actions is currently in preclinical development [4]. We are focused on another aspect of their putative application in translation medicine, namely, on enzymes associated with EVs from various bacterial species for cancer therapy. The present study aimed to identify and characterize *Pseudomonas aeruginosa* ATCC 10145 and *Komagataeibacter obedience* IMBG180 proteases associated with EVs and demonstrate their effect on *in vitro* human malignant cells. **Methods.** The proteogenomic *in silico* analysis of the bacterial proteases in bacterial genomes involved using cutting-edge tools from the Bacterial and Viral Bioinformatics Resource Center. We detected the secretory proteases using the SignalP 6.0 program and determined the localization of proteases in membranes using ProtComp Version 9. EVs from the secretome of bacteria were isolated using the ExoBacteria Isolation Kit (System Biosciences, USA). We confirmed the morphology of the proteolytic positive extracellular vesicles using transmission electron microscopy. The size distributions of OMVs were determined by analyzing the intensity of light scattering. We assessed the cell viability after the effect of EVs using the thiazolyl blue tetrazolium bromideassay and researched apoptosis rates in human cell lines using the Annexin V analysis. **Results.** The *in silico* proteogenomic and functional analyses present the first identification and characterization of 13 protease genes in *P. aeruginosa*and, four genes in *K. obedience* genomes. The subcellular localization of proteases associated with EVs was predicted. Proteolytic-positive EVs exhibited cytotoxic effects against malignant human cell lines, resulting in apoptosis. **Conclusion.** *In silico* proteogenomic analysis of the EV-associated proteases, along with functional studies, contribute to our understanding of the proteolytic activity of EVs and the potential applications of these nanostructures in biomedicine. **Fundings.** This work was supported by the Simons Foundation Support Grant 1290589.

K e y w o r d s: nanovesicles, membrane-associatedproteases, apoptosis, cancer cells.

REFERENCES:

1. *Mobarak H et al.* Prokaryotic microvesicles Ortholog of eukaryotic extracellular vesicles in biomedical fields. *Cell Communication and Signaling*. 2024; **22**(1): 80.
2. *Micoli F et al.* Outer Membrane Vesicle Vaccine Platforms. *BioDrugs.* 2024; **38**:47–59.
3. *Jiang C et al.* Bacterial outer membrane vesicles as drug delivery carrier for photodynamic anticancer therapy. *Front Chem.* 2023; **11**:1284292.
4. *Liu H et al.* Unveiling clinical applications of bacterial extracellular vesicles as natural nanomaterials in disease diagnosis and therapeutics. *Acta Biomaterialia.* 2024; **180**:18–45.