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Short communication

Toxic potential of *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin: *in silico* investigation of adverse outcomes in cancer patients

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Introduction

Pseudomonas aeruginosa mannose - sensitive hemagglutinin (PA-MSHA) is a biologic drug that has immunomodulating function when used in tumor adjuvant therapy (Zhang et al., 2019). It has been suggested that PA-MSHA can serve as a new anticancer treatment, with cytotoxic activity in different human cancer types, including, but not limited to breast and bladder cancer (Xu et al., 2014; Zhu et al., 2013). Proposed mechanisms include anti-proliferative effects on cancer cells by affecting regulation of the cell cycle and inducing apoptosis via caspase 3 and caspase 9 signaling. Moreover, when combined with a tyrosine kinase inhibitor, PA-MSHA was reported to both induce caspase-3/caspase-9 cleavage and increase inhibition of epidermal growth factor receptor (EGFR)-dependent signaling, leading to better suppression of cancer cells proliferation (Zhao et al., 2016).

However, its harmful and possibly toxic effects remain unknown. Thus, in the present study, we aimed to investigate the potential of PA-MSHA to induce adverse outcomes in cancer patients by *in silico* gene data-mining.

Materials and methods

The Pubmed.gov database (https://pubmed.ncbi.nlm.nih.gov/ approached on 27/5/2022) was searched manually for publications containing either *Pseudomonas aeruginosa* mannose - sensitive hemagglutinin, or *Pseudomonas aeruginosa* MSHA, or PA-MSHA. A total of 93 papers were retrieved - 9, 45, and 39, respectively. After excluding reports written in language other than English, non-relevant studies, and duplicates, 24 publications were systematically scanned with an aim to select the genes impacted by the given bacteria.

Selected down- and upregulated genes were analyzed with a set of computational tools to investigate the role of PA-MSHA-induced genes in the gene ontology processes and molecular pathways and furher decipher toxic potential of PA-MSHA in cancer patients. Tools used for investigation were: GeneMania, ToppGene ToppFun, and Comparative Toxicogenomics Database (CTD). GeneMania (https://genemania.org/ approached on 28/5/2022) was used to identify other genes related to a set of input genes based on genetic interactions, pathways, co-expression, co-localization and protein domain similarity. ToppGene ToppFun (https://toppgene.cchmc.org/enrichment.jsp approached on 28/5/2022) is a web portal for gene list enrichment analysis that helps in identifying top molecular functions, biological processes, cellular components, and pathways enriched by detected gene set. Finally, CTD Set Analyzer tool (http://ctdbase.org/ approached on 30/5/2022) was used to retrieve gene set-based enriched diseases (corrected $p<0.01$).

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Results and discussion

The comprehensive scanning of selected literature revealed 64 genes influenced by PA-MSHA among which 16 were downregulated and 48 upregulated. GeneMania network analysis of repressed genes predicted 20 related genes, as well as 391 tight links between them. Majority of interactions (38.93%) were physical protein-protein interactions, while predicted relations where the second most common (18.71%). The ToppGene ToppFun function listed top 10 biological processes ($p<0.01$) for 36 highlighted and downregulated genes among which were: response to hormone, regulation of G1/S transition of mitotic cell cycle, and protein phosphorylation. Moreover, pathways in cancer, p53 signaling pathway, and PI3K-Akt signaling were recognized as the most enriched for the analyzed set of genes. Finally, CTD tool retrieved 236 diseases that were associated with PA-MSHA repressed genes, such as: digestive system diseases (28 annotated genes), skin and connective tissue diseases (23 annotated genes), endocrine system diseases (19 annotated genes), and immune system diseases (19 annotated genes).

The same analysis steps were repeated with the set of PA-MSHA-upregulated genes. Generated gene-gene interactions networks showed that 28.97% of the genes were co-expressed, while 19.87% were in physical interactions. The gene ontology examination showed that top enriched biological processes ($p<0.01$) were related to cellular response to molecule of bacterial origin, biotic stimulus or lipopolysaccharides, as expected. Toll-like receptor signaling pathway with Toll Like Receptor 4 (TLR4) Cascade, and Toll-like receptor signaling related to MyD88 were reported as the most significant pathways linked to investigated set of genes. Among diseases, infections, immune system, skin and connective tissue, as well as respiratory tract diseases might have a central role in PA-MSHA induced adverse outcomes.

As expected, a phase II clinical study reported that PA-MSHA has a good safety profile, with only 6.2% patients discontinuing treatment due to neutropenia, leukopenia, and thrombocytopenia, as well as fever, skin induration at the injection site, and rash (Lv et al., 2015). This can be explained by pro-inflammatory activity of PA-MSHA described in the conducted study. Moreover, it was observed that PA-MSHA has the potential to inhibit the production of TNF-α, increase IL-10 levels and promote the generation of CD4+CD25+Foxp3+ T cells (Liu et al., 2013), which might explain the predicted PA-MSHA-induced immune system diseases.

Conclusion

In the present study, 64 PA-MSHA-interacting genes were identified. In-depth bioinformatic analysis might propose that PA-MSHA cause immune-related side effects due to the immune-stimulatory mechanisms, such as response to molecule of bacterial origin and Toll-like receptor signaling pathway as well as via downregulation of PI3K-Akt signaling what needs further *in vitro* and *in vivo* investigation.

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References

Liu, X.F., Wang, L., Qu, Y., Zhong, D.W., Miao, X.Y., Yao, H.L., 2013. Effect of the PA-MSHA vaccine on septic serum-induced inflammatory response. Mol. Med. Rep. 7(4), 1350-1354. https://doi.org/10.3892/mmr.2013.1337

Lv, F., Cao, J., Liu, Z., Wang, Z., Zhang, J., Zhang, S., Wang, L., Zhao, X., Shao, Z., Wang, B., Hu, X., 2015. Phase II study of *Pseudomonas aeruginosa*-mannose-sensitive hemagglutinin in combination with capecitabine for Her-2-negative metastatic breast cancer pretreated with anthracycline and taxane. PLoS One 10(3), e0118607. https://doi.org/10.1371/journal.pone.0118607

Xu, W.H., Liu, Z.B,, Houm, Y.F., Hong, Q., Hu, D.L., Shao, Z.M., 2014. Inhibition of autophagy enhances the cytotoxic effect of PA-MSHA in breast cancer. BMC Cancer 14, 273. https://doi.org/10.1186/1471-2407-14-273

Zhao, X.M., Pan, S.Y., Huang, Q.L., Lu, Y.N., Wu, X.H., Chang, J.H., Liu, Z.B., Cai, X.W., Liu, Q., Wang, J.L., Fu, X.L., 2016. PA-MSHA in combination with EGFR tyrosine kinase inhibitor: a new strategy to overcome the drug resistance of non-small cell lung cancer cells. Oncotarget 7(31), 49384-49396. https://doi.org/10.18632/oncotarget.9891

Zhang, W., Sun, J., Shen, X., Xue, Y., Yuan, S., Wang, X., 2019. Effect of PA-MSAH preprocessing on the expression of TLR-4-NF-κB pathway and inflammatory factors in the intestinal tract of rats with septic shock. Exp. Ther. Med. 17(4), 2567-2574. https://doi.org/10.3892/etm.2019.7247

Zhu, Y.P., Bian, X.J., Ye, D.W., Yao, X.D., Zhang, S.L., Dai, B., Shen, Y.J., 2013. *Pseudomonas aeruginosa*-mannose-sensitive hemagglutinin inhibits proliferation and induces apoptosis in a caspase-dependent manner in human bladder cancer cell lines. Oncol. Lett. 5(4), 1357-1362. https://doi.org/10.3892/ol.2013.1201