Strszczenie w języku angielskim rozprawy pt. „Gronkowcowe systemy toksyna-antytoksyna w badaniach omicznych''

Staphylococcal toxin antitoxin system in omics research

Staphylococcus aureus is an opportunistic pathogen of humans and animals. Up to 50-60% of the human population is temporarily colonized by *S. aureus*. Under conditions of homeostasis, these bacteria live on the mucous membranes and skin of the host without causing infections. After breaking through the external body tissues, bacteria being able to cause infection. *S. aureus* is the etiological factor of inflammatory diseases of the skin and subcutaneous tissues and bacteremia. Particularly dangerous and difficult to eradicate make *S. aureus* their increasing antibiotic resistance and the ability to synthesize numerous virulence factors, as well as the developed adaptive mechanisms that determine survival, among which toxin-antitoxin (TA) systems play an important role.

TA systems are small genetic elements widely distributed in bacteria, which are encoded in both plasmids and bacterial chromosomes. Eight types of TA systems have been identified. They are composed of two molecules, a toxin and an antitoxin. The mechanism of activation of TA systems involves disturbing the balance between the amount of toxin and antitoxin in the cell. TA systems are responsible for the maintenance of plasmids, influence the response of cells to stress, regulate gene expression and promote a phenotype change to biofilming or "persister" cells. Types I and II TA systems have been described in *S. aureus*, but the function of some of them remains unclear.

The study examined the influence of the type I SprG1/SprF1 and type II PemK/PemI TA systems on changes in Staphylococcus aureus gene expression using proteomics techniques: two-dimensional - differential gel electrophoresis (2D-DIGE), mass spectrometry, and transcriptomics: next-generation sequencing, real time polymerase chain reaction.

Before undertaking 2D-DIGE studies, several methods of staphylococcal protein precipitation were compared. The method using the TRI-Reagent was chosen as the best due to the highest efficiency and the best separation of protein spots in the polyacrylamide gel.

Studies on the SprG1/SprF1 system demonstrated the influence of SprF1 antitoxin on changes in the expression of several genes in cells. Global inhibition of translation and reduction of gene expression under the influence of antitoxin allow us to postulate its possible role in the formation of cells with the "persisters" phenotype. Removal of the SprG1/SprF1

system cassette correlated with a reduction in protein secretion from cells. The SprG1 toxin is most likely responsible for the reduced secretion. As the proteins secreted outside the cells of pathogenic bacteria include virulence factors (clumping factor B, protein A, glutamyl endopeptidase, staphopain B), the reduced secretion outside the cells with the simultaneous absence of the toxin may suggest a link of this TA system with the virulence of *S. aureus.*

The next part of the work was a comparative analysis of the impact of two homologous toxins of the PemK/PemI systems. $PemK_{Sa}$ and $PemK_{Sd}$ toxins have endoribonuclease activity, but differ in toxicity towards *S. aureus* cells, which results from differences in the sequence of both proteins. Gene expression observed at the transcriptome level changes over time; in bacteria expressing $PemK_{Sd}$, the observed changes disappear, while those with $PemK_{Sa}$ intensify. The $PemK_{Sd}$ toxin changes the level of some genes, leading to the overexpression of most of the affected, but it does not affect the cell growth rate. It is therefore unlikely that the PemKSd toxin is involved in the global regulation of gene expression, and its function remains to be elucidated. The second toxin, $PemK_{Sa}$, significantly alters the expression of more than one third of *S. aureus* genes related to transport and metabolism, translation, ribosome biogenesis, virulence, and many other cellular processes. This results in deregulation of metabolism and slower cell growth. The activity of the $PemK_{Sa}$ toxin also leads to a reduction in protein secretion from the cell, which may suggest a link between this toxin and the regulation of virulence.

The obtained results expand the state of knowledge about staphylococcal TA systems and may constitute the basis for further research.