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Trastuzumab plant biosimilar for breast cancer treatment: new approaches for the production of antibodies against the HER2/neu positive cells

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In the modern world one of the promising areas of pharmacology is the production of monoclonal antibodies against various diseases, in particular against cancer. In our laboratory a technology for the production of trastuzumab plant biosimilar (TPB) was developed. Trastuzumab is an antibody targeted against cancer cells that characterized by the presence of HER2/neu oncoprotein on their surface. We have proved that the ability of TPB to inhibit proliferation of cells comparable to the commercial therapeutic antibody - Herceptin. The vector encoding the processed TPB (pTPB) was also constructed and it consisted of light and heavy chains of antibody, connected together by a linker - kex2p protease recognition site. pTPB was demonstrated to be capable of inhibiting cancer cell proliferation, nevertheless, its activity appeared to be lower comparing to Herceptin. The partial loss of pTPB functional activity could be explained by the presence of 20-30% amount of the unprocessed variant in the examined samples.

The aim of this work was to study the principles of obtaining plant-made biosimilars and improve the developed platform for their production. The following tasks were completed: (1) the construction of the vector encoding pTPB under control of two novel promoters; (2) the study of the pTPB maturation in plant cell and the properties of unprocessed pTPB polypeptide.

It is known that kex2p-like protease is localized in the Golgi apparatus (GA). We suggested that some amount of the synthesized pTPB polypeptide does not go through the GA, thus could not be cleaved by kex2p-like protease. However, our results have not confirmed this suggestion. We founded that the unprocessed pTPB contains an N-linked glycan, which indicates that the protein passed through the GA.

Thus, the presence of the unprocessed pTPB polypeptide might be explained by the insufficient amount of the internal kex2p-like protease in the cell during the overexpression of the target pTPB gene. So we proposed that the additional production of that protease could lead to the increase in the cleavage efficiency. To identify and clone potential kex2p-like protease we performed bioinformatics analysis and chose several candidate genes from N.benthamiana and N.tabacum. We plan to co-express those genes with pTPB-encoding construct to improve antibodies' yield.

Finally, we estimated the expression levels of pTBP under control of two novel promoters that we have recently identified and isolated from N.benthamiana: the promoter of the gene encoding Kunitz peptidase inhibitor-like protein (NbKPILP) and the promoter of the gamma-thionin gene. Both new promoters appeared to be less effective comparing to 35S-promoter mediating pTPB production. It is noteworthy that the ratio of the unprocessed pTPB form was almost the same, despite the level of production, thus we concluded that it does not depend on the capacity of promoters.

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