

Abstract

The rapid development of the gene engineering techniques, especially methods for *in vitro* directed evolution and combinatorial mutagenesis, has triggered the generation of new binding agents to almost any antigen of interest as an alternative to broadly used antibodies. These so-called non-Ig scaffolds are often derived from proteins with useful biophysical properties. While the therapeutic market is still dominated by monoclonal antibodies, the easy option of desired customization of non-Ig binders by conventional methods of gene engineering predestine them largely for the use in the diagnostic area.

The ABD scaffold, derived from a three-helix bundle of albumin-binding domain of streptococcal protein G, represents one of the small non-Ig scaffolds. In our laboratory, we have established a highly complex combinatorial library developed on the ABD scaffold. This ABD scaffold-derived library was used to generate unique binders of human prostate cancer (PCa) biomarkers PSP94, KLK2, KLK11 for the more precise diagnosis of PCa.

The second part of the thesis describes the generation of ABD-derived binders selectively recognizing different phenotypes of circulating tumor cells as a binding component of the cell capture zone of microfluidic chip for lung adenocarcinoma diagnosis. Beside this already proven model, the newly developed libraries derived from the Myomedin scaffold were used to yield a novel type of binders that would recognize other cell-surface epitopes on target molecules.

In the third part of the thesis, a collection of unique protein binders targeted to the Shiga toxin (Stx) B-subunit was generated with the use of ABD-derived combinatorial library. They were optimized for the surface display in *Lactococcus lactis* and functionally characterized. After subsequent improvement of the binding properties of particular S1B variants, Lactic acid bacteria with surface-displayed S1B binders would be useful for antagonizing pathogenic bacteria strains by the removal of Stx from the human gastrointestinal tract.

The last part of the thesis is devoted to the development of unique polyvalent vaccines for immunization of piglets and calves against enteric diseases. Current vaccines offer only a limited number of valences and do not offer protection against some serious diarrheal diseases. For successful introduction of the vaccines to the market, precise and reliable ELISA kits and protocols for verification and validation of their quality were developed. This assay is able to detect serum antibodies against 10 factors of the pathogenicity and is currently being used as a crucial part of the validation process before these highly innovative vaccines enter the market in 12 European countries.