

Generation and evaluation of IgY antibodies specific to Tumor Associated NADH Oxidase (tNOX) and its selected epitopes

mgr inż. Maria Łęcka

The main topic of this PhD thesis is related to generation of IgY antibodies specific towards cancer antigen and their further application in cancer diagnostics.

The dissertation thesis begins with the introduction section, where structure and function of ENOX2 protein and IgY antibodies are described as well as their potential application in design of diagnostic tests.

Subsequently study motivation and research goals are presented, which includes 1) hens' immunization with recombinant ENOX2 protein and conjugates of carrier proteins with ENOX2 specific peptides, followed by isolation of IgY antibodies from egg yolk, 2) characterization of generated IgY antibodies, 3) detection of ENOX2 in cancer cell lines and cancer patient samples with specific IgY antibodies and the design of diagnostic test for detection of ENOX2 in biological samples.

The next, most extensive chapter describes and discusses the obtained results. First part outlines *in silico* analysis of ENOX2 sequence and structure, based on which ENOX2 epitopes for animal immunization were selected. In the experimental chapter animals' immunization with recombinant ENOX2 protein and conjugates of carrier proteins with ENOX2 specific peptides is described followed by IgY isolation from egg yolk using precipitation method. Interaction of all generated IgY antibodies with the antigen used for immunization as well as recombinant ENOX2 protein is reported. Furthermore, titer and detection limit of IgY's with ELISA and Western blot were determined. In the next part of the chapter covalent modifications of IgY antibodies and their use in detection of ENOX2 in cancer cell lines and cancer patients' serum samples are reported. Modified IgY antibodies are used for design and initial tests of diagnostic test based on sandwich ELISA, both as a capture and detection agents. At the end of experimental chapter immunohistochemical analysis of cancer patient specimens performed with IgY antibodies is presented.

The last chapter of PhD describes the most important experimental methods, which were used to characterize generated IgY antibodies (ELISA, Western blot, fluorescence microscopy and immunohistochemistry).

In conclusion, it was shown that the IgY antibodies generated towards ENOX2 epitopes are specifically recognizing not only antigen used for immunization but also recombinant ENOX2 protein. All generated IgY antibodies were characterized in terms of their specificity, titer, and antigen detection limit. Moreover, IgY antibodies were successfully used in design of prototype of diagnostic test for ENOX2 detection. Unfortunately, due to ambiguous results from fluorescence microscopy and immunohistochemistry analysis utility of IgY antibodies in those techniques was not proven.