Abstract

The cytochromes P450 are enzymes participating in metabolism of endogenous and exogenous compounds. Their substrates include also carcinogens which may initiate carcinogenesis after activation by CYP450. Inductors of these enzymes are also chemopreventive compounds which are very popular and recommended in current time. Thus, studying of the effect of the chemopreventive compounds on cytochromes P450 induction and cancer development is of a high clinical importance.

The CYPs are most commonly found in the liver. However, there are forms that have not been detected in any human healthy tissue but their overexpression was observed in tumors. For this reason, they could serve for diagnosis and prognosis of cancer. Among these cytochromes are CYP2S1 and 2W1 which can be prognostic markers of colorectal cancer. Therefore, it would be opportune to have some tools for these enzyme detection.

One option is immunodetection of cytochromes P450 by Western blot using the specific antibodies. Today mammalian antibodies (IgG) are the most widely used but antibodies isolated from egg yolk (IgY) become popular mainly due to the large number of undisputed advantages.

For the preparation of the peptide immunogen, suitable peptide sequences were selected from CYP2S1 and 2W1 primary structure. The synthesized peptides were then conjugated with the carrier protein KLH and the hens were immunized by these conjugates. Then IgY from egg yolk were isolated by the extraction and precipitation methods with sodium chloride. The ability of antibodies to recognize the peptide was demonstrated by ELISA. The next procedure was purification by affinity chromatography when the specific antibodies were obtained. Finally the purified antibodies were used for the immunodetection of CYP2S1 and 2W1 in the biological material. CYP2S1 and 2W1 were showed in the lysates from cell lines MT-3, A549, MRC-5 and MCF-7 by Western blot technique. The cytochromes P450 in these samples were not detected probably due to their small amount. Another sample for immunodetection was the suspension of *E. coli* with the plasmid for expression of CYP2S1. The expressed CYP2S1 was detected well by the prepared antibodies. (In Czech)