Preparation of IgY antibodies specific to human tumor markers

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Abstract

The aim of the study was generation and biochemical evaluation of IgY antibodies specific for human tumor markers. The obtained IgY antibodies were further used for the development of highly specific and sensitive prototypes of immunoenzymatic and lateral assays for potential application in early cancer detection and monitoring of the treatment progress.

Immunoglobulins Y, in contrast to mammalian antibodies routinely used in diagnostic assays, represent an interesting diagnostic agents since they lack reactivity with rheumatoid factor, human anti-mouse antibodies and complement activation. Moreover, specific IgY antibodies can be obtained in high yield from egg yolks after hens immunization which eliminates the animal bleeding process. In addition, due to the phylogenetic distance between mammals and birds it is possible to generate highly specific immunoglobulins Y against conserved mammalian epitopes which production may be impossible for conventional IgG generation.

In order to obtain highly specific antibodies for human tumor makers, hens were immunized with the native proteins and their selected epitopes conjugated to the carrier proteins followed by IgYs isolation from egg yolks. The highly immunogenic epitopes were selected using the computer-assisted epitope prediction tools. Isolated antibodies were subjected to detailed biochemical analysis including the determination of purity, avidity maturation, titer and target antigen detection limit. It is worth to highlight that IgY antibodies specific to the selected peptidyl epitopes demonstrated a cross-reactivity with native protein markers. In order to improve their diagnostic potential, IgY antibodies were further purified using different affinity chromatography techniques.

Subsequently, the obtained IgY antibodies were used for the development of an immunoassays for the detection of cancer-specific markers, in an sandwich ELISA format applying mouse monoclonal IgG as a capture antibody and chicken polyclonal IgY as a detection antibody (IgG/IgY), sandwich ELISA that relies exclusively on chicken IgY antibodies (IgY/IgY) and an immunochromatographic assays also functioning in the IgG/IgY and IgY/IgY format. For the construction of lateral flow devices, gold nanoparticles functionalized with generated marker-specific IgY antibodies were utilized as an visualizing agent.