Abstract

Transmissive spongiform encephalopathies (TSEs) are neurodegenerative diseases characterized by depositions of abnormally folded prion protein (PrP^{TSE}) in brain. PrP^{TSE} is at present the only specific biochemical marker of human and animal TSEs. Diagnostic tests are based on the detection of PrPres after proteinase K digestion of brain homogenate using Western blot or on the immunohistochemistry of fixed brain tissue, which are both difficult and time consuming. In this work we focused on development of a new type of tests based on PrP detection without need of proteinase K digestion.

As deposits of PrP^{TSE} remain in the body for a long time, there is a substantial chance of them being nonenzymatically modified by glycation. The detection of glycated PrP^{TSE} may have a potential to serve as a diagnostic marker. We prepared monoclonal antibodies specific for carboxymethyl lysine/arginine modified prion protein. Bacterially expressed and purified recombinant human prion protein (rhPrP) was modified by glyoxylic acid that introduces carboxymethyl groups on lysine and arginine residues present within the molecule of the protein. Modified rhPrP (rhPrP-CML) was used for immunization of laboratory mice and hybridoma cells were prepared. Screening of cell supernatants resulted in the selection of 4 promising clones. One of them (EM-31) strongly reacts with human and mouse recombinant PrP-CML and three other clones react also with CML *in vitro* modified human and mouse brain PrP.

Next we focused on development of DELFIA based assay for a quick and sensitive detection of the GPI-anchorless prion protein fragment, named PrP226*, in human brain tissue homogenates. By calculating the ratio between the signals of native and denatured samples applied to the assay we were able to observe significant difference between TSE affected brains and control brains. In the present study we show that the PrP226* fragment accumulates in prion aggregates and after being released from them by a denaturation procedure, it could serve as a proteinase K digestion independent biomarker for human TSEs.

Keywords: Transmissible Spongiform Encephalopathies, Creutzfeldt-Jakob disease, GSS, Prion, V5B2, Immunoassay, DELFIA, Anchorless PrP, PrP226* fragment, Proteinase K