Panel of monoclonal antibodies to GCP2 for cancer diagnosis

Protein GCP2 forms with gamma-tubulin complexes that are essential for microtubule nucleation. It has been shown that gamma-tubulin is often overexpressed in cancer cells and thus represents a new marker for cancer prognosis. It was reported that GCP2 is aberrantly expressed in gliomas. It was suggested that overexpression of gamma-tubulin complex proteins leads to deregulation of microtubules in cancer cells. Monoclonal antibodies, prepared in the Institute of Molecular Genetics, AS CR v.v.i., specifically recognize mouse GCP2 in immunoblotting. In attempt to determine the range of their applicability, specificity and reactivity of antibodies in the other immunological assays was determined in the frame of TACR gamma project. Epitope sequences were also determined. The results revealed that mouse monoclonal antibodies GCP2-02 (IgG1, kappa), GCP2-03 (IgG1, kappa), GCP2-04 (IgG2b, kappa) and GCP2-05(IgG1, kappa) are suitable for ELISA tests (**Fig. 1A and 1B**), immunoblotting (**Fig. 2A and 2B**), and immunoprecipitation, mainly GCP2-04 (**Fig. 2C**). Antibodies label GCP2 both in immunofluorescence microscopy (**Fig. 3)** and on tissue sections (GCP2-04). Antibody-binding regions on GCP2 molecule was determined by pulldown experiments (**Fig. 4A**). Epitope mapping with immobilized synthetic peptides led to more precise localization of the targeted epitopes (**Fig. 4B**). Antibodies recognize three unique epitopes in the N-terminal domain of GCP2 protein (**Fig. 4C**).

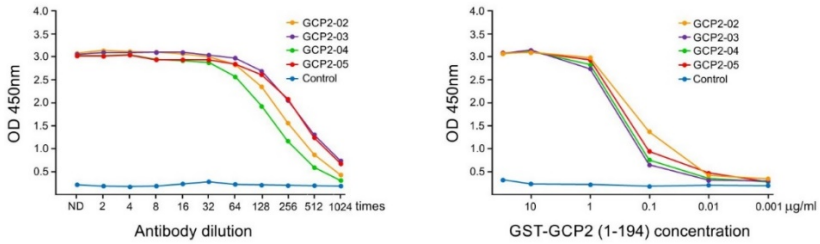

Fig. 1. ELISA tests with anti-GCP2 antibodies. (A) Titration of antibodies in the form of hybridoma supernatants on the immobilized GCP2 protein. Domain, GST-GCP2 (1-194), containing targeted epitopes and negative control (GST; Control) were immobilized at the concentration 10 µg/ml. ND, non-diluted antibody. Binding to GST is shown only for GCP2-02 (B) Detection of the immobilized GST-GCP2 protein using panel of antibodies to GCP2 and a negative control antibody to tubulin (Control). Antibodies were used in the form of undiluted supernatants.

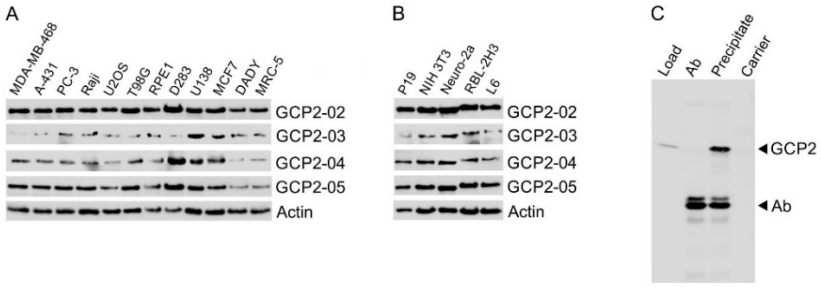

Fig. 2. Analysis of GCP2 expression in cell lines (A-B) using antibodies GCP2-02, GCP2-03, GCP2-04 and GCP2-05, and its immunoprecipitation (C) using GCP2-04. (A) Immunoblot analysis of total cell lysates from human cell lines solubilized in SDS-sample buffer. (B) Immunoblot analysis of total cell lysates from mouse (P19, NIH 3T3, Neuro-2a) and rat (RBL-2H3, L6) cell lines. Actin, loading control. (C) Immunoprecipitation from human U2OS extract (1% NP-40). Ab, immobilized antibody without extract; Carrier, protein G without antibody incubated with extract. GCP2-04 was used both for immunoprecipitation and immunoblotting.

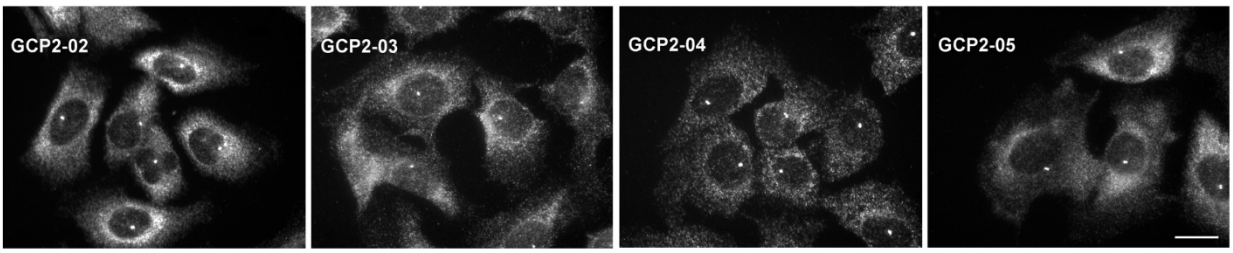

Fig. 3. Immunofluorescence localization of GCP2 in interphase human U2OS cells using monoclonal antibodies GCP2-02, GCP2-03, GCP2-04 and GCP2-05. The antibodies detect GCP2 concentrated in centrosomes. Fixation by methanol. Scale bar: 20 µm.

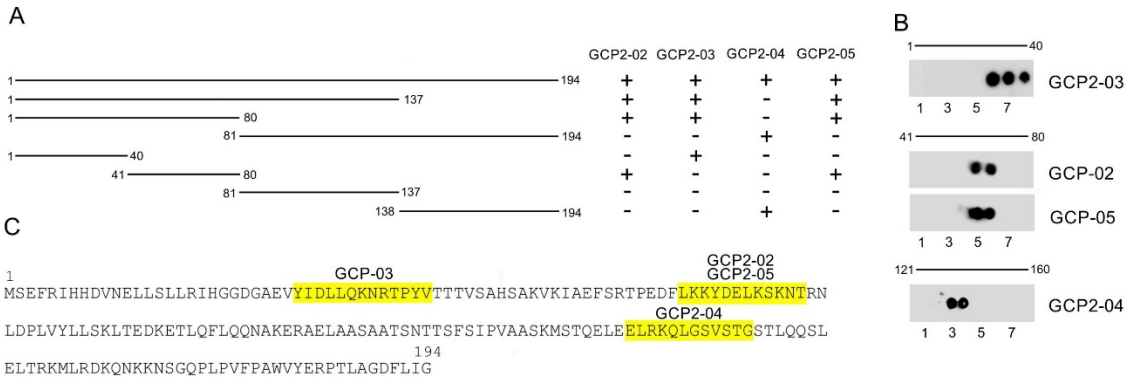

Fig. 4. Identification of epitopes recognized by antibodies GCP2-02, GCP2-03, GCP2-04 and GCP2-05. (A) Analysis of antibody reactivities with GST-constructs of GCP2 protein (+, positive reaction). (B) Epitope mapping on immobilized overlapping synthetic peptides (15-mers, overlap 11 aa) covering the aa regions 1-40, 41-80, and 121-160. (C) Positions of the epitopes recognized by antibodies GCP2-02, GCP2-03, GCP2-04 and GCP2-05 are indicated by yellow color.

Conclusions: The results show that mouse monoclonal antibodies GCP2-02 (IgG1, kappa), GCP2-03 (IgG1, kappa), GCP2-04 (IgG2b, kappa) and GCP2-05 (IgG1, kappa) exhibit properties, which predestine them for ELISA tests, immunoblotting, immunoprecipitation (GCP2-02, GCP2-04, GCP2-05), immunocytochemical and histochemical (GCP2-04) experiments. Epitope sequences recognized by antibodies were identified.

To get more information about the GCP2-02, GCP2-03, GCP2-04 or GCP2-05 antibodies or to buy nonexclusive licence for hybridoma cells producing the antibodies, please contact **Center for Technology Trasfer**, IMG AS CR, Videnska 1083, 14220 Praha 4, Czech Republic; Tel. (420-241 063 227 or 420-602 892 876).