SophoMer F10 in the Characterization of the Monoclonal Antibody GCP3-01 Targeting the GCP3 protein

Microtubules, composed of αβ-tubulin heterodimers, are essential for vital cellular processes and are nucleated from from microtubule-organizing centers, such as centrosomes. γ-Tubulin, together with γ-tubulin complex proteins (GCP2, GCP3, GCP4, GCP5 and GCP6), forms γ-tubulin ring complexes (γ-TuRCs), which play a critical role in microtubule nucleation [1]. γ-Tubulin has been shown to be frequently overexpressed in cancer cells, and abnormal expression of GCP2 and GCP3 has been reported in gliomas [2]. It is proposed that overexpression of γ-TuRC proteins contributes to microtubule dysregulation in cancer cells [3].

To study microtubules and their nucleation, the following unique **mouse monoclonal antibodies (mAb) with well-defined epitope locations** were prepared at the Laboratory of Biology of Cytoskeleton, IMG CAS, Prague, Czech Republic (https://dbc.img.cas.cz/antibodies.html): anti-α-tubulin mAb **TU-01** (IgG1, epitope α65-79; Merck SAB4700970) [4, 5], anti-β-tubulin mAb **TU-06** (IgM, epitope β81-95; Merck SAB4700971) [6, 7], anti-γ-tubulin mAb **TU-30** (IgG1, epitope γ439-444 ; Merck SAB4701012) [8, 9], and anti-GCP2 mAb **GCP2-02** (IgG1, epitope at aa 64-75; Merck MAPT1322) [2]. Recently, a new anti-GCP3 mAb, **GCP3-01** (IgG1, epitope at aa 14-23), was added to this portfolio and thoroughly characterized [10].

SophoMer F10 (Sophomer s.r.o.) is a fully synthetic material designed to replace bovine serum albumin in immunology assays. Here, we present typical results from ELISA and immunoblot experiments using SophoMer F10 during the characterization of mAb **GCP3-01.** This mAb was raised against a GST (glutathione S-transferase)-tagged fragment encoding the human GCP3 polypeptide (amino acids 1-310; UniProtKB - Q96CW5).

ELISA

The assay was performed in high binding 96-well half-area polystyrene plates (Corning Inc, Cat. No. 3690). All washing steps throughout the assay (four washes per step) were carried out using Tris buffer solution (TBS; 10 mM Tris-Cl, pH 7.4, 150 mM NaCl) containing 0.05% Tween 20 (TBST). GST-GCP3(1-310) or GST alone, were immobilized at concetration 10 µg/ml in PBS (10 mM phosphate, pH 7.4, 150 mM NaCl) for 2 hours at 37°C. After washing, the plates were blocked by incubation with **0.1% SophoMer F10 in TBS** for 1 hour at room temperature (RT).The plates were then washed and incubated with the primary mAbs, GCP3-01 or TU-01, in the form of hybridoma supernatants diluted in **0.05% SophoMerF10 in PBS** for 1hour at RT. Following washing, the plates were incubated with a secondary goat anti-mouse IgG antibody conjugated with horseradish peroxidase (Millipore, Cat. No.; AP 124P) diluted 1:250 in **0.05% SophoMer F10 in TBST** for 1 hour at RT. After final washing step, the plates were incubated with 3,3',5,5'-tetramethylbenzidine (TMB) Liquid Substrate (Sigma-Aldrich, Cat. No. T8665). The reaction was stopped after 11 min by adding Stop Reagent for TMB Substrate (Sigma-Aldrich, Cat. No. S5814). Absorbace was measured at 450 nm on Sunrise plate Reader (TECAN).

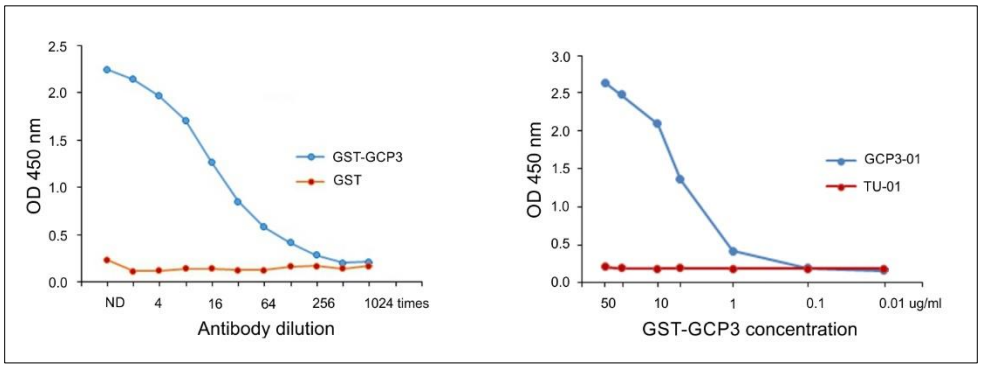

Fig. 1. Reactivity of the GCP3-01 antibody in ELISA assays using SophoMer F10. (**A**) Titration of the GCP3-01, applied as hybridoma supernatant, on immobilized GST-GCP3(1-310) protein and a negative control protein (GST), both at concentration of 10 μg/ml. ND indicates undiluted antibody. (**B**) Detection of immobilized GST-GCP3(1-310) at various concentrations using the GCP3-01 antibody and a negative control antibody (TU-01, targeting α-tubulin). Both antibodies were applied as undiluted hybridoma supernatants.

IMMUNOBLOTTING

Proteins separated by SDS-PAGE were transferred onto 0.45 μm Protran nitrocellulose membranes (Amersham, Cat. No. 10600002) by electroblotting. All washing steps throughout the assay (three washes per step) were performed using TBST. After blotting, the nitrocellulose membranes were blocked with **0.4% SophoMer F10 in TBST** for 30 minutes at room temperature. The membranes were then incubated overnight at 4°C with either the primary mAb GCP3-01 targeting GCP3 or the newly prepared mAb GST-01 targeting GST [10], both applied as undiluted hybridoma supernatants. Following washing, the membranes were incubated with a secondary goat anti-mouse antibody conjugated to horseradish peroxidase (Promega Biotec, Cat. No. W402B) diluted 1:10,000 in 2% non-fat dry milk in TBST. After additional washing, the peroxidase signal was detected using the SuperSignal WestPico Chemiluminescent Substrate (Pierce, Cat. No. 34080) and visualized using the the LAS 3000 imaging system (Fujifilm).

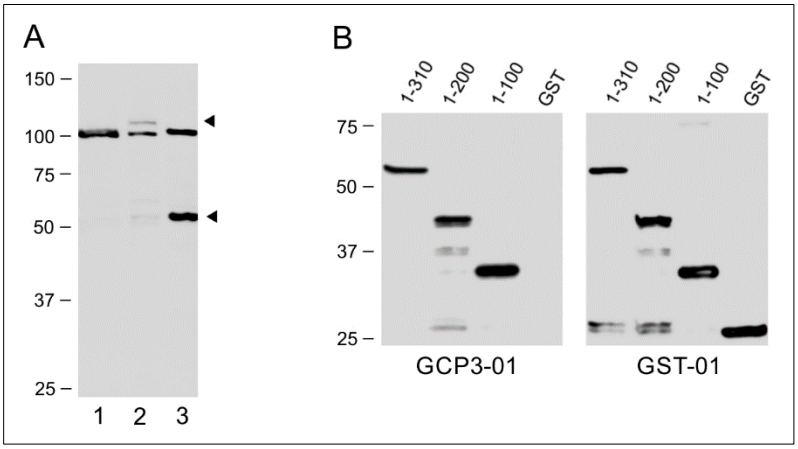

Fig. 2. Reactivity of the GCP3-01 antibody in immunoblot experiments using SophoMer F10. (A) Detection of GCP3 (103 kDa) in whole-cell lysates from mouse bone marrow-derived mast cells (BMMCL, *lane 1*), rat basophilic leukemia cells (RBL-2H3, *lane 2*), and human glioblastoma (U-251 MG, *lane 3*). Arrowheads indicate the positions of GCP3 isoforms. (B) Analysis of GCP3-01 antibody reactivity with GST-tagged GCP3 protein fragments. The epitope is localized in the N-terminal domain of GCP3 (amino acids 1-100). The anti-GST antibody (GST-01) marks the positions of GST-tagged proteins.

CONCLUSIONS

The results demonstrated that **SophoMer F10** can fully replace bovine serum albumin in ELISA and immunoblotting assays on nitrocellulose membranes.

The mAb GCP3-01 targets the GCP3 protein and is suitable for ELISA (Fig. 1A-B), and for immunoblotting experiments, where it reacts with GCP3 (103 kDa) and its isoforms in total cell lysates from different cell lines (Fig. 2A). The epitope recognized by GCP3-01 is located in the N-terminal domain of the molecule (amino acids 1-100), as shown in Fig. 2B. Further characterization through pull-down experiments with GST fragments of this region, followed by epitope mapping on immobilized synthetic pepetides, identified the precise position of the epitope as the sequence at amino acid position 14-23 of human GCP3. GCP3-01 is highly specific and does not interact with other GCP family proteins (GCP2, GCP4, GCP5, GCP6). An alignment of amino acid sequences revealed that the GCP3-01 epitope is phylogenetically highly conserved from human to the African clawed frog (*Xenopus laevis*). **GCP3-01 (IgG1, kappa) is the first antibody with a known precise localization of its epitope** [10].

The mAb GST-01 (IgG2b, kappa) possesses properties that make it suitable for immunoblotting, ELISA, and immunoprecipitation as described [10].

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