Supplementary materials and methods

Preparation of monoclonal antibody to FKBP8

Glutathione-S-transferase-fused human FKBP8 (GST-FKBP8) was expressed in *Escherichia coli* strain JM109 transformed with pGEX-4T3 containing FKBP8 gene. GST-FKBP8 was purified with Glutathione-conjugated Sepharose Affinity Matrix (Amersham Pharmacia Biotech, Franklin Lakes, NJ). Purified GST- FKBP8 was immunized to *Balb/c* mouse. Lymphonodus cells were obtained after 5 boost immunizations and were fused to mouse myeloma PAI cells. The resulting hybridomas were screened by enzyme-linked immuno-sorbent assay using GST and GST-FKBP8. The selected clones were further screened by flow cytometry using 293T cells expressing HA-FKBP8 (O'Reilly et al., 1998). Among several positive clones, two clones strongly reactive to human FKBP8 were designated as KDM-11 and 19 (IgG2b). Antibodies were purified from supernatants of cell culture by Protein G Sepharose 4B beads (Amersham).

Preparation of recombinant proteins

His_6-tagged FKBP8 (His-FKBP8) and thioredoxin-fused NS5A (aa 25-213, domain I) (Trx-NS5A) were generated from recombinant *Escherichia coli*. Either pET30a encoding FKBP8 or pET32a encoding NS5A (aa 25-213) was introduced into *E. coli* strain BL21(DE3). Ten milliliter of overnight culture was added into 1 L of 2 x YT medium and was incubated at 37℃. When the absorbance of culture supernatant indicated 0.4 OD_{600}, isopropyl beta-thiogalactoside (IPTG) was added at final concentration of 0.4 mM and was then incubated at 20℃ overnight. After centrifugation, the cell pellet was washed once with 10 ml phosphate buffered saline (PBS). The washed cell pellet was suspended in 40 ml lysis buffer (50mM phosphate buffer [pH 8.0] containing 150mM NaCl, 1% Triton X-100 and 0.2 μg/ml lysozyme) and was incubated at 4℃ for 2h. After freezing and thawing, the mixture was sonicated at 4℃ for 5 min and was treated with 0.02 mg/ml of DNase at room temperature for 5 min. The cell lysates were centrifuged at 10,000 x g for 5 min. The resulting supernatant

was mixed with 0.5 ml of Nickel agarose beads (Sigma, St. Louis, MO) and was rotated at 4°C for 60 min. The Nickel beads were washed twice with PBS containing 10 mM imidazole. The recombinant protein was eluted from Nickel beads with PBS containing 0.25 M imidazole. Bovine Hsp90 was purchased from Sigma. Bovine Hsp90-α shares 99.5% amino acid identity to human Hsp90-α.

Reference

O'Reilly, L.A., Cullen, L., Moriishi, K., O'Connor, L., Huang, D.C. and Strasser, A. (1998) Rapid hybridoma screening method for the identification of monoclonal antibodies to low-abundance cytoplasmic proteins. *Biotechniques*, **25**, 824-830.