Monoclonal Antibodies Register

New Monoclonal Antibodies Specific for Microtubule-Associated Protein MAP2

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Background

The microtubule-associated protein 2 (MAP2) is an abundant neuronal cytoskeletal protein that binds to tubulin and stabilizes microtubules (Herzog and Weber, 1978). MAP2 is essential for the development and maitenance of neuronal morphology (Matus, 1991). In neurons MAP2 occurs as three primary isoforms, the high molecular weight MAP2a, MAP2b, and the low molecular weight MAP2c, which result from alternative splicing of the MAP2 gene (Chung et al., 1996). The low molecular weight isoform, MAP2c, is expressed in developing brain and is down-regulated during brain maturation, whereas the high molecular weight MAP2b is expressed in both developing and adult brain. The MAP2a appears only after brain maturation (Tucker, 1990). All these forms bind to microtubules through a domain near its carboxyl terminus that contains either three or four similar repeats of a 31-amino-acid motif (Lewis et al., 1988). MAP2 together with MAP4 and tau proteins belong to the family of thermostable proteins associated with microtubules.

Description of the antibodies MT-03 and MT-04

Production

The hybridoma cell line producing the MT-03 antibody was obtained after immunization of a Balb/c mouse with a thermostable fraction of porcine brain microtubule protein prepared by two temperature-dependent cycles of polymerization/depolymerization (MTP-2) (Shelanski et al., 1973), and subsequent fusion of the spleen cells with Sp2/0 myeloma cells. The hybridoma cell line producing the MT-04 antibody was obtained after immunization of a Balb/c mouse with a pellet of porcine brain cold-stable proteins after depolymerization of microtubules, and subsequent fusion of the spleen cells with Sp2/0 myeloma cells. Details of the fusion, screening by ELISA, cloning and production of ascitic fluid have been described previously (Viklický el al., 1982; Dráber et al., 1988).

Specificity

Immunoblotting analysis performed with the total extract of porcine brain or MTP-2 showed that both antibodies reacted with proteins with relative electrophoretic mobility corresponding to the position of high molecular weight MAP2 isoforms (280 kDa) and with their proteolytic fragments. No cross-reaction was observed with other proteins, including tubulin subunits and tau proteins (Fig.1), and the secondary antibody alone gave no staining (not shown). When thermostable fractions of MTP-2

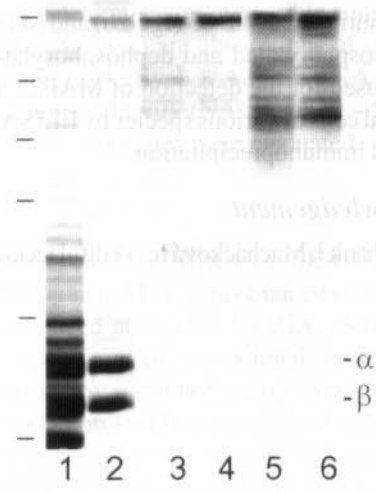

Fig. 1. Specificity of antibodies MT-03 and MT-04 to MAP2 as determined by immunoblotting. Total extract of adult porcine brain (lanes 1,3,5) and microtubule protein MTP-2 (lanes 2,4,6) were separated on 6% polyacrylamide gel. Lanes 1–2, Comassie Blue staining. Lanes 3–6, immunoreactivity with antibodies MT-03 (lanes 3–4) and MT-04 (lanes 5–6). Bars on the left margin indicate the position, from top to bottom, of specific molecular weight markers (280 kDa, 205 kDa, 116 kDa, 97.4 kDa, 66 kDa and 45 kDa); α- and β- denote the position of tubulin subunits.

Received January 17, 2000. Accepted January 21, 2000.

This work was supported by the grant EU 1450 from the Ministry of Education of the Czech Republic and by the grant 312/96/K205 from the Grant Agency of the Czech Republic.

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Abbreviations: MAP2 – microtubule-associated protein 2, MTP-2 – microtubule protein prepared by two temperature-dependent polymerization/depolymerization cycles.

containing MAP2a, MAP2b and tau proteins were immunoblotted, the antibodies reacted only with MAP2a and MAP2b proteins and their fragments (not shown). Both antibodies precipitated MAP2a and MAP2b from the whole brain extracts, as confirmed by immunoblotting with the MT-01 antibody against MAP2 (Riederer et al., 1995). Each of the two antibodies reacted with the same set of proteolytic fragments in total brain extract, MTP-2 and thermostable fraction of MTP-2. The antibodies differed one from the other in the reactivities with corresponding proteolytic fragments. This indicates that the antibodies recognize different antigenic determinants. The antibodies can bind dephosphorylated MAP2 prepared by the treatment of pig brain extract with alkaline phosphatase from *Escherichia coli* using a standard procedure (Shaw et al., 1986). In the control sample, treated in exactly the same way except that the phosphatase was omitted, no increase in proteolysis of a 280 kDa protein was apparent. In contrast, the control NF-01 antibody, recognizing a phosphorylated epitope on NF-H (Lukáš et al., 1993), did not react with the phosphatase-treated sample. On immunoblots of total cell extract of non-neuronal cell lines 3T3 (mouse embryonal fibroblasts) and rat basophilic leukemia (RBL), the antibody MT-04 gave no staining, whereas the antibody MT-03 reacted with a thermolabile 220 kDa protein (not shown). The common antigenic determinants for MAP2 and microtubule-associated proteins in non-neuronal cells were previously described (Dráberová et al., 1986; Wiche et al., 1986).

Properties

The antibodies MT-03 (IgG2b) and MT-04 (IgG1) can bind phosphorylated and dephosphorylated MAP2 and can be used for the detection of MAP2a and MAP2b in neuronal cells of various species by ELISA, immunoblotting and immunoprecipitation.

Acknowledgement

We thank I. Macháčková for skillful technical assistance.

References

Chung, W. J., Kindler, S., Seidenbecher, C., Garner, C. C. (1996) MAP2a, an alternatively spliced variant of microtubule-associated protein 2. *J. Neurochem.* **66,** 1273-1281.

Dráber, P., Lagunowich, L. A., Dráberová, E., Viklický, V., Damjanov, I. (1988) Heterogeneity of tubulin epitopes in mouse fetal tissues. *Histochemistry* **89,** 485-492.

Dráberová, E., Dráber, P., Viklický, V. (1986) Cellular distribution of a protein related to neuronal microtubule-associated protein MAP-2 in Leydig cells. *Cell Biol. Int. Rep.* **10,** 881-890.

Herzog, W., Weber, K. (1978) Fractionation of brain microtubule-associated proteins. Isolation of two different proteins which stimulate tubulin polymerization in vitro. *Eur. J. Biochem.* **92,** 1-8.

Lewis, S. A., Wang, D., Cowan, N. J. (1988) Microtubule-associated protein MAP2 shares a microtubule binding motif with tau protein. *Science* **242,** 936-939.

Lukáš, Z., Dráber, P., Buček, J., Dráberová, E., Viklický, V., Doležel, S. (1993) Expression of phosphorylated high molecular weight neurofilament protein (NF-H) and vimentin in human developing dorsal ganglia and spinal cord. *Histochemistry* **100,** 495-502.

Matus, A. (1991) Microtubule-associated proteins and neuronal morphogenesis. *J. Cell Sci.* **15** (Suppl.), 61-67.

Riederer, B. M., Dráberová, E., Viklický, V., Dráber, P. (1995) Changes of MAP2 phosphorylation during brain development. *J. Histochem. Cytochem.* **43,** 1269-1284.

Shaw, G., Osborn, M., Weber, K. (1986) Reactivity of a panel of neurofilament antibodies on phosphorylated and dephosphorylated neurofilaments. *Eur. J. Cell Biol.* **42,** 1-9.

Shelanski, M. L., Gaskin, F., Cantor, C. R. (1973) Microtubule assembly in the absence of added nucleotides. *Proc. Natl. Acad. Sci. USA* **70,** 765-768.

Tucker, R. P. (1990) The roles of microtubule-associated proteins in brain morphogenesis: a review. *Brain Res. Rev.* **15,** 101-120.

Viklický, V., Dráber, P., Hašek, J., Bártek, J. (1982) Production and characterization of a monoclonal antitubulin antibody. *Cell Biol. Int. Rep.* **6,** 725-731.

Wiche, G., Herrmann, H., Dalton, J. M., Foisner, R., Leichtfried, F. E., Lassmann, H., Koszka, C., Briones, E. (1986) Molecular aspects of MAP-1 and MAP-2: microheterogeneity, in vitro localization and distribution in neuronal and nonneuronal cells. *Ann. N.Y. Acad. Sci.* **466,** 180-198.