Histochem Cell Biol (1998) 109:231–239
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ORIGINAL PAPER

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Expression of class III β-tubulin in normal and neoplastic human tissues

Accepted: 29 July 1997

Abstract The class III β-tubulin isotype is widely used as a neuronal marker in normal and neoplastic tissues. This isotype was, however, also immunodetected in certain tumours of non-neuronal origin such as squamous cell carcinoma. Using a newly described monoclonal antibody we compared the distribution of class III β-tubulin in normal and neoplastic tissues. The TU-20 mouse monoclonal antibody was prepared against a conserved synthetic peptide from the C-terminus of the human class III β-tubulin isotype, and its specificity was confirmed by immunoblotting, by competitive enzyme-linked immunosorbent assay and by immunofluorescence microscopy on cultured cells. In different cell lines of various origins the antibody reacted only with neuroblastoma Neuro-2a cells and with embryonal carcinoma P19 cells stimulated to neuronal differentiation by retinoic acid. Immunohistochemistry on formaldehyde-fixed paraffin-embedded normal human tissues revealed the presence of the class III β-tubulin isotype in cell bodies and processes of neuronal cells in the peripheral and central nervous systems. In other tissues, this β-tubulin isotype was not immunodetected. Class III β-tubulin was found in all cases of ganglioneuroblastoma, ganglioneuroma, medulloblastoma, neuroblastoma, sympathoblastoma and in one case of teratoma. In contrast, no reactivity was detected in tumours of non-neuronal origin, including 32 cases of squamous cell carcinoma. The results indicate a specific TU-20 epitope expression exclusively in neuronal tissues. The antibody could thus be a useful tool for the probing of class III β-tubulin functions in neurons as well as for immunohistochemical characterisation of tumours of neuronal origin.

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Introduction

A major building component of microtubules is the 100-kDa protein, tubulin, which consists of two 50-kDa subunits designated α and β. Both tubulin subunits are encoded by a family of genes that produce polypeptides differing primarily in the C-terminal variable domain comprised of 15 amino acids. In vertebrates, seven β-tubulin isotype classes were identified on the basis of highly conserved variable domains and characteristic cell type distribution (Sullivan 1988). It is still an unresolved issue whether all β-tubulin isotype classes are functionally equivalent or whether some are unique in specifying the functional properties of microtubules in particular tissues (Ludueña 1993). The neuron-associated class III β-tubulin isotype is most abundant in cells of neuronal origin (Burgoyne et al. 1988) but was also immunodetected in Sertoli cells of the testis (Lewis and Cowan 1988) and transiently in non-neuronal embryonic tissues (Lee et al. 1990b). Using the monoclonal antibody TuJ1 (Lee et al. 1990b), which is widely used as a marker of class III β-tubulin, it was shown that this isotype was one of the earliest appearing markers of neuronal differentiation (Easter et al. 1993). Class III β-tubulin was expressed in a differentiation-dependent manner in human neuroblastic tumours, highlighting neoplastic neuritogenes is in medulloblastomas (Katsetos et al. 1989), retinoblastomas (Katsetos et al. 1991) and peripheral neuroblastomas (Katsetos et al. 1994). Class III β-tubulin can be used as a marker for primitive neuroepithelium in teratomas (Caccamo et al. 1989). A corresponding epitope was, however, also immunohistochemically detected on paraffin sections of human squamous cell carcinomas, lymphomas and melanomas (Scott et al. 1990).

In this study we have used a newly prepared monoclonal antibody, raised against an eight-amino acid peptide from the C-terminal end of human class III β-tubulin, in order to evaluate the immunohistochemical distribution of this isotype in normal and neoplastic human tissues, including squamous cell carcinomas.

Materials and methods

Cells

Human epidermoid carcinoma line A431, human lung carcinoma line LEP 19, human cervical adenocarcinoma (HeLa cells), mouse neuroblastoma Neuro-2a, mouse embryonic 3T3 fibroblasts, mouse embryonal carcinoma P19, rat leukaemic basophilic granulocyte RBL-1 and rat kangaroo kidney PtK_2 were from the bank of cell lines of the Institute of Molecular Genetics, Praque. Human squamous cell carcinoma lines UM SCC 1, UM SCC 2 and UM SCC 22B were kindly provided by T.E. Carey (University of Michigan, Ann Arbor, USA). Cells were grown on coverslips at 37° C in air with 5% CO_2 in Eagle's minimal essential medium supplemented with 10% (v/v) heat-inactivated fetal calf serum (Biocom, Opava, Czech Republic). P19 cells were stimulated to neuronal differentiation by incubation for 5 days with 1 µM all *trans*-retinoic acid (Sigma-Aldrich, Prague, Czech Republic).

Tissue samples

Human tissues were obtained as surgical specimens. All specimens were biopsies, except autopsy samples of spinal cord (boy 9 years, bronchopneumonia), peripheral nerve (male 77 years, bronchopneumonia), cerebellar cortex (boy 9 years, leukaemia) and cerebral cortex (boy 2 years, congenital heart disease). Post-mortem time for all autopsy specimens was 5 h. Samples were either snap frozen in a mixture of propane–butane, precooled in liquid nitrogen and stored at –70° C, or fixed in buffered formaldehyde for 24–48 h and routinely embedded in paraffin for histopathological classification. Samples of normal and neoplastic tissues were from the Pathology Department of The Netherlands Cancer Institute and from the Pathology Department of the Medical Faculty of Masaryk University, Brno.

Peptide synthesis

An eight-amino acid peptide, ESESQGPK, corresponding to the human class III β-tubulin sequence 441–448 (Sullivan and Cleveland 1986) was synthesised. Cysteine (C) has been added to the N-terminus of the peptide in order to allow oriented coupling to carrier proteins. In competitive assays, an eight-amino acid peptide, GEEEGEEY, corresponding to the porcine α-tubulin sequence 444–451 (Ponstingl et al. 1981) and a 16-amino acid peptide, EYHAATRPDYISWGTQ, corresponding to the human γ-tubulin sequence 434–449 (Zheng et al. 1991), were also used. The peptides were prepared by I. Bláha (Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague) by the solid-phase method (Merrifield 1963) on the classic Merrifield's chlormethylated carrier. Details of peptide synthesis and HPLC purification were described previously (Viklický et al. 1988).

Protein preparation

Microtubule proteins from porcine brain were preparred by two temperature-dependent cycles of assembly and disassembly according to Shelanski et al. (1973). Tubulin depleted of microtubule-associated proteins was obtained by phosphocellulose chromatography (Weingarten et al. 1975). Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin (BSA; Serva Feinbiochemica, Heidelberg, Germany) as standard. Tubulin samples were reduced and carboxymethylated (Crestfield et al. 1963), dialysed against 0.06 M TRIS-phosphate sample buffer containing 0.8 M sucrose and stored in liquid nitrogen.

Antibodies

Peptide (C)441–448 was covalently coupled to maleimide-activated keyhole limpet hemocyanin (KLH) or BSA (Imject Activated Immunogen Conjugation kit; Pierce, Rockford, USA) at a ratio of 2 mg peptide/2 mg activated carrier protein according to the manufacturer's directions. A Balb/c mouse was immunised subcutanously (s.c.) with 100 µg of KLH with coupled peptide in Freund's complete adjuvant. Booster injections of 100 µg of antigen in Freund's incomplete ajduvant were given s.c. at 3-week intervals. Sera were monitored for antibody activity by enzyme-linked immunosorbent assay (ELISA) on BSA with coupled peptide. The fusion with mouse myeloma Sp2/0 cells, screening by ELISA, cloning and production of ascitic fluids have been described previously (Viklický et al. 1982; Dráber et al. 1988).

Monoclonal antibodies TU-12 (Dráber et al. 1991) and TU-14 (Dráber et al. 1989) against β-tubulin were described previously. It was shown earlier that in brain the antibody TU-14 reacted with β-tubulin classes I, II, IV, but not with the neuron-specific class III (Linhartová et al. 1992). A new mouse monoclonal antibody, TU-18 (IgM), against αβ-tubulin dimers was selected from hybridomas producing antibodies against cytoskeletal proteins extracted from human gingival epithelium. In double-label fluorescence, microtubular structures were detected with an affinity-purified polyclonal antibody against the αβ-tubulin dimer (Dráber et al. 1991). Fluroescein isothiocyanate (FITC)-conjugated anti-mouse Ig antibody as well as horseradish peroxidase-conjugated anti-mouse Ig antibody were from Sevac (Prague, Czech Republic) or from Dako (Glostrup, Denmark); tetramethylrhodamine isothiocyanate (TRITC)-conjugated anti-rabbit Ig antibody was from Sanbio (Uden, The Netherlands) and anti-mouse Ig antibody conjugated with alkaline phsophatase was purchased from Promega Biotec (Madison, USA).

ELISA

Competitive ELISA was performed as described (Grimm et al. 1987) using HPLC-pure peptides, BSA and prediluted supernatants. Values for the maximal optical density were between 0.8 and 0.9. Antibodies were mixed with variable concentrations of peptides or BSA, and incubated for 60 min at room temperature. The remaining antibody-binding activity was detected in ELISA using immobilised phosphocellulose-purified porcine brain tubulin. Bound antibodies were detected with anti-mouse antibody conjugated with horseradish peroxidase and *o*-phenylenediamine as chromogen. Optical density at 490 nm was measured with a Microelisa Mini Reader (Dynatech Laboratories, Alexandria, USA). Three measurements were made for each test point. Standard errors of the means of triplicate absorbance measurements were less than 3%.

Electrophoresis and immunoblotting

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli (1970). Separated proteins were transferred onto nitrocellulose sheets by electroblotting (Towbin et al. 1979). Details of the immunostaining procedure using secondary antibody labelled with alkaline phosphatase are described elsewhere (Dráber et al. 1988). Monoclonal antibodies were used as ascitic fluids diluted 1:1000. Isoelectric points (pIs) were determined by isoelectric focusing in a horizontal thin layer with the help of pI markers (Pharmacia LKB Biotechnology, Bromma, Sweden).

Immunofluorescence on cell lines

Fixed cytoskeletons were immunostained as described (Dráber et al. 1989). Briefly, cells grown on coverslips were extracted for 1 min with 0.2% Triton X-100 at 37° C and fixed for 20 min in 3% formaldehyde at the same temperature. Alternatively, cells were fixed for 10 min in methanol at −20° C followed by 6 min in acetone at −20° C. Monoclonal antibodies were used as undiluted supernatants or ascitic fluids diluted 1:500. For double-label immunofluorescence with affinity-purified rabbit antibody against the αβ-tubulin dimer and monoclonal antibodies, the coverslips were first incubated with monoclonal antibody and, after washing, with polyclonal antibody (dilution 1:10). Slides were incubated thereafter simultaneously with TRITC-conjugated anti-rabbit Ig antibody and FITC-conjugated anti-mouse Ig antibody.

Immunohistochemical staining

Cryosections were fixed in cold methanol/acetone or in formaldehyde followed by Triton X-100 treatment (Lukáš et al. 1993). Paraffin sections were cut, deparaffinised and the endogenous peroxidase was blocked as described (Van Bommel et al. 1994). The 5-µm-thick sections were then heated in a microwave oven in 10 mM citrate buffer, pH 6.0 for a few seconds until boiling and then allowed to cool over a period of 15 min. Immunostaining with antibodies was performed as described (Ivanyi et al.1990). Samples were incubated for 1 h with monoclonal antibody (ascitic fluid diluted 1:100), washed and then incubated with either FITC-conjugated anti-mouse antibody (dilution 1:100) or peroxidase-conjugated anti-mouse antibody (dilution 1:50). 3,3´-Diaminobenzidine was used as the chromogen in immunoperoxidase staining and Harris's haematoxylin was used for counterstaining the nuclei. In negative controls, the primary antibodies were omitted from the staining procedures.

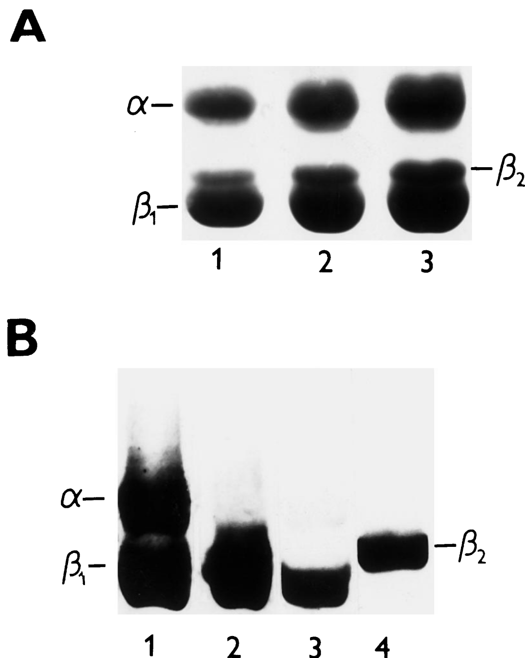

Fig. 1A, B Reactivity of monoclonal antibodies with reduced and carboxymethylated porcine brain tubulin. **A** Coomassie blue staining. *Lanes 1–3* contain 15 µg, 20 µg and 30 µg of protein, respectively. **B** Immunoblot with monoclonal antibodies. *Lanes 1–4* immunoreactivity with antibodies TU-18, TU-12, TU-14 and TU-20, respectively; 30 µm protein per lane; 7.5% SDS-polyacrylamide gel. β_1 and β_2 denote the electrophoretic mobility of the major and minor (class III) β-tubulin components, respectively

Results

Specificity of antibody TU-20

The mouse monoclonal antibody TU-20 (IgG1, pI 6.4–6.6) used in this study was raised against the phylogenetically conserved synthetic peptide from the C-terminal end of class III β-tubulin. To verify the specificity of the antibody, immunoblotting was performed on reduced and S-carboxymethylated purified porcine brain tubulin, which can be separated by SDS-PAGE into α-tubulin, major β-tubulin (β_1) and minor β-tubulin (β_2) components. β_2 has lower relative electrophoretic mobility than β_1 as demonstrated in Fig. 1A. It is known that β_2 contains class III β-tubulin (Banerjee et al. 1990). The antibodies TU-18, TU-12 and TU-14 were used as markers of separated tubulins. Antibody TU-18 reacted with α-tubulin, β_1 and β_2 components; antibody TU-12 with both β_1 and β_2 components; and antibody TU-14 with the β_1 component (Fig. 1B, lanes 1–3). The antibody TU-20 stained only the β_2 component (Fig. 1B, lane 4). In whole-cell lysate of porcine brain, TU-20 reacted only

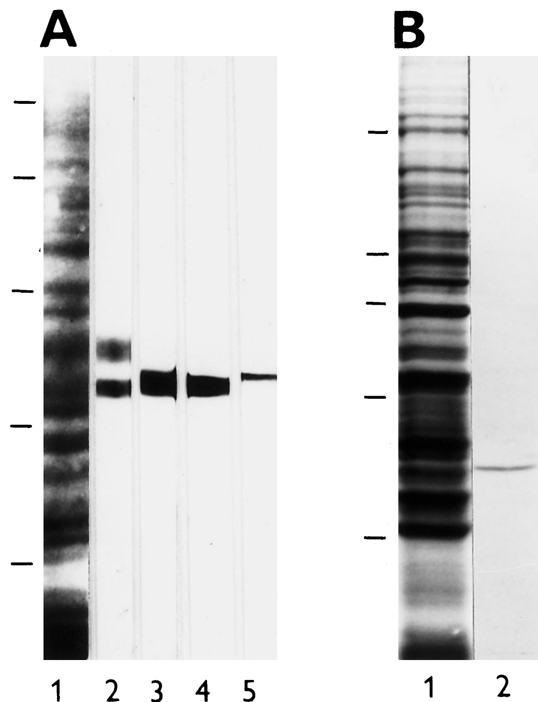

Fig. 2A, B Specificity of antibodies determined by immunoblotting. **A** Immunoblot of total extract of porcine brain with anti-tubulin monoclonal antibodies. *Lane 1* amido black staining of transferred proteins. *Lanes 2–5* immunoreactivity with antibodies TU-18, TU-12, TU-14 and TU-20, respectively; 5–15% gradient SDS-polyacrylamide gel. *Bars* on the *left* margin indicate positions, from top to bottom, of specific molecular weight markers (116, 97.4, 66, 45 and 29 kDa). **B** Immunoblot of total extract of neuroblastoma Neuro-2a cells with TU-20. *Lane 1* Coomassie blue staining. *Lane 2* immunoreactivity with antibody TU-20; 7.5% SDS-polyacrylamide gel. *Bars* on the *left* margin indicate positions, from top to bottom, of specific molecular weight markers (205, 116, 97.4, 66 and 45 kDa)

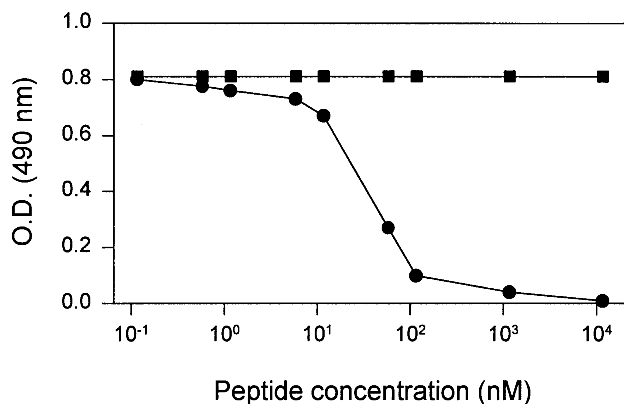

Fig. 3 Comparison of the reactivity of TU-20 antibody with peptides by indirect competitive ELISA. (● C-terminal peptide, ESESQGPK, of class III β-tubulin, ■ C-terminal peptide, GEEEGEEY, of tyrosinated α-tubulin)

with a protein of electrophoretic mobility corresponding to β-tubulin and no cross-reactivity with other proteins was detected. In comparison with the marker antibodies TU-12 and TU-14, the TU-20 antibody reacted with a minor band in the β-tubulin region (Fig. 2A). From the panel of cell lines of different tissue origin tested, TU-20 reacted only with neuroblastoma Neuro-2a cells (Fig. 2B). It did not react in immunoblotting with any proteins from the following cell lines: 3T3, A431, HeLa, LEP 19, P19, PtK_2, RBL-1, UM SCC 1, UM SCC 2 and UM SCC 22B.

The specificity of the antibody was further confirmed by competitive ELISA. The antibody was strongly inhib-

Fig. 4A–F Specificity of TU-20 antibody determined by immunofluorescence. Double-label staining of mouse neuroblastoma Neuro-2a cells (**A, B**), human squamous cell carcinoma UM SCC 2 (**C, D**) and mouse embryonal P19 carcinoma cells stimulated to neuronal differentiation by retinoic acid (**E, F**) with affinity-purified polyclonal anti-tubulin antibody (**A, C, E**) and monoclonal antibody TU-20 (**B, D, F**), *Bar* 20 μm

ited by the C-terminal peptide, ESESQGPK, of class III β-tubulin that was used for immunisation, however, the C-terminal peptide of α-tubulin, GEEEGEEY, as well as C-terminal peptide EEFATEGTDRKDVFFY of γ-tubulin did not exert any inhibitory effect at the highest concentration tested (10 μM). Similarly, control BSA was without effect at that concentration. A typical example of competitive ELISA is shown in Fig. 3.

Immunofluorescence experiments on the panel of cell lines tested showed decoration of all microtubule structures – interphase arrays, mitotic spindle, midbody in late telophase – in neuroblastoma cells (Fig. 4A, B). In other cell lines, including squamous cell carcinoma cell lines UM SCC 1, UM SCC 2 (Fig. 4C, D) and UM SCC 22B, no specific staining was detected. In a population of P19 cells stimulated to neuronal differentiation, the antibody TU-20 strongly stained only those cells with neuronal processes and not the undifferentiated cells (Fig. 4E, F). Microtubules were detected in preparations extracted with Triton X-100 and fixed with formaldehyde as well as in preparations fixed with cold methanol/acetone.

Immunolocalisation of class III β-tubulin in normal human tissues

On both methanol/acetone- and formaldehyde-fixed frozen sections, as well as on formaldehyde-fixed paraffin-embedded material, the antibody reacted with a staining pattern characteristic for nerve fibres and neuronal cells. Immunostaining was stronger on formaldehyde-fixed material. On formaldehyde-fixed paraffin-embedded material the antibody intensively stained neural processes and ganglion cells in grey matter of the spinal cord (Fig. 5A) and in

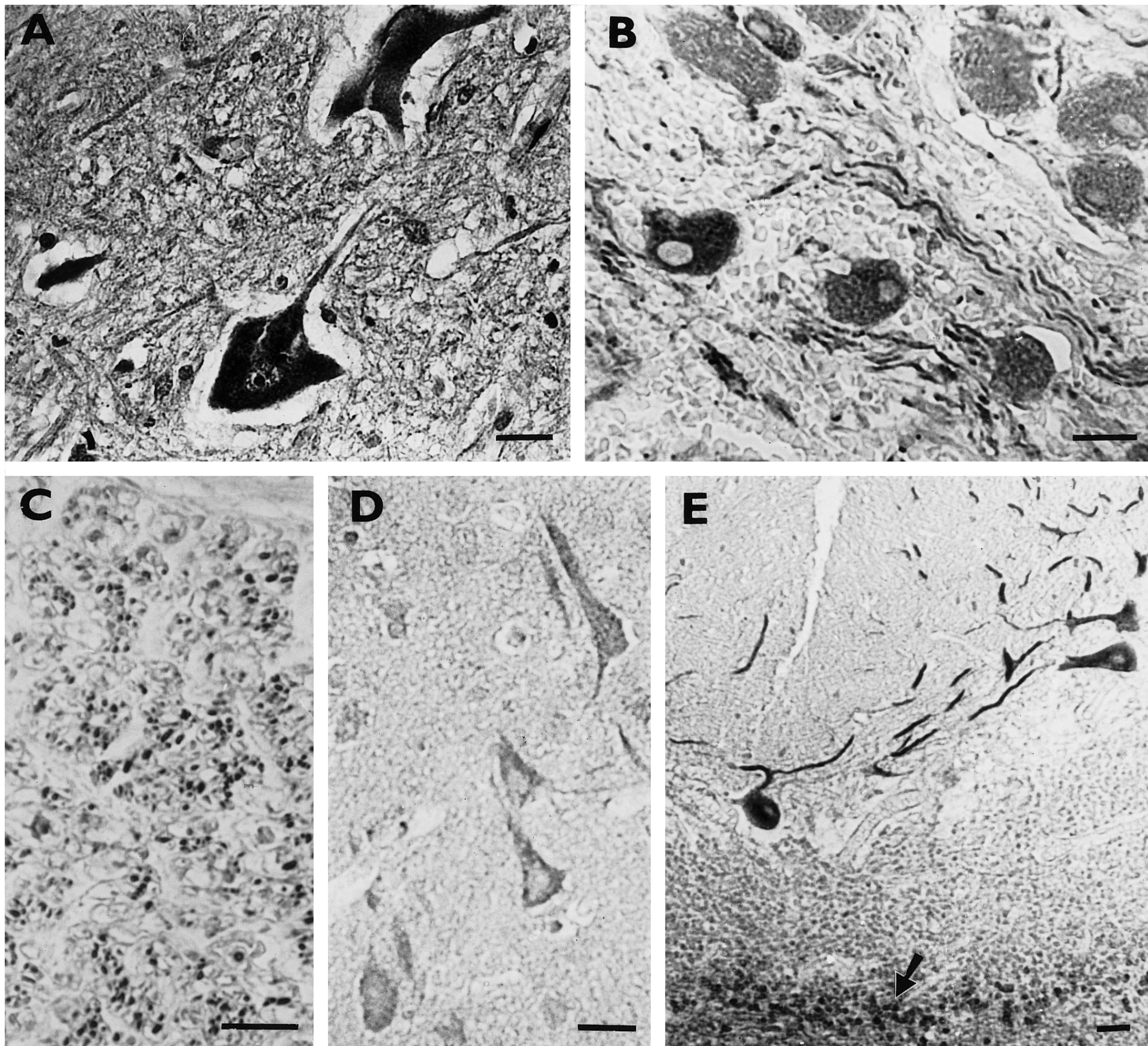

Fig. 5A–E Immunohistochemical distribution of class III β-tubulin in different brain regions and peripheral nerves detected by staining with TU-20 antibody. Immunoperoxidase staining of multipolar ganglion cells and neuronal processes in the ventral horn of grey matter in the lumbar part of the spinal cord (**A**), ganglion cells and their processes in sympathetic ganglion (**B**), axons in nervus femoralis (**C**), perikarya of pyramidal cells in cerebral cortex (**D**) and Purkinje cells and bodies of granule cells (*arrow*) in the internal granular layer of cerebellum (**E**). *Bar* 20 μm

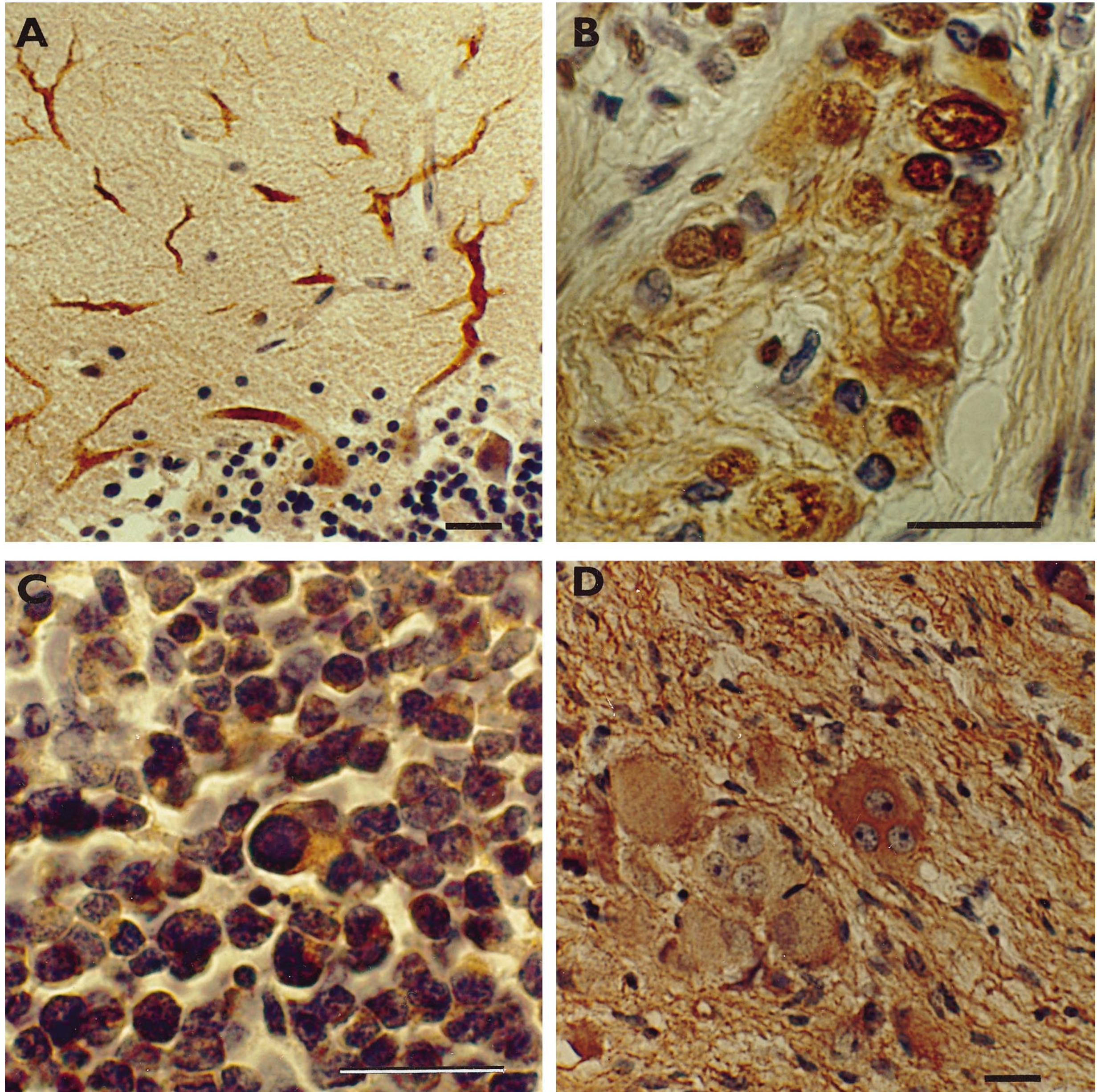

Fig. 6A–D Immunohistochemical distribution of class III β-tubulin in cerebellum, large intestine, medulloblastoma and ganglioneuroma. Immunoperoxidase staining of perikarya and dendrites of Purkinje cells in cerebellum (**A**), perikarya and neuronal processes in Auerbach's plexus in large intestine (**B**), perikarya of neoplastic cells in medulloblastoma (**C**) and neuronal processes and perikarya of neoplastic ganglion cells in ganglioneuroma (**D**). *Bar* 20 μm

sympathetic ganglia (Fig. 5B). Strong staining was detected in nerve fibres of peripheral nerves, as demonstrated on the cross-section of nervus femoralis (Fig. 5C). No staining of surrounding myelin sheets was found. Mainly the perikarya of pyramidal cells were moderately strongly stained in grey matter of the cerebral cortex (Fig. 5D). Purkinje cells were most intensively stained in cerebellum (Fig. 5E). Heterogeneous staining of granule cells bodies was found in the internal granular layer (IGL), with strong positivity in the part of the IGL adjacent to white matter (Fig. 5E). In the medullar layer, axons were stained. In the molecular layer of the cerebellum, only dendrites of Purkinje cells were strongly decorated (Fig. 6A); parallel fibres and basket cells remained unstained. Overall, glial elements of the nervous system were unstained. In the large intestine, lung, skin (inclusive of sweat glands, sebaceous glands), salivary gland, tongue and urinary bladder no reactivity was detected, except in peripheral nerves. A faint staining of Sertoli cells was occasionally detected in testis. An example of staining of perikarya and neuronal processes in Auerbach's plexus in the large intestine is shown in Fig. 6B.

Immunolocalisation of class III β-tubulin in neoplastic tissues

The TU-20 antibody heterogeneously stained perikarya of cells in neuroblastomas, sympathoblastoma and med-

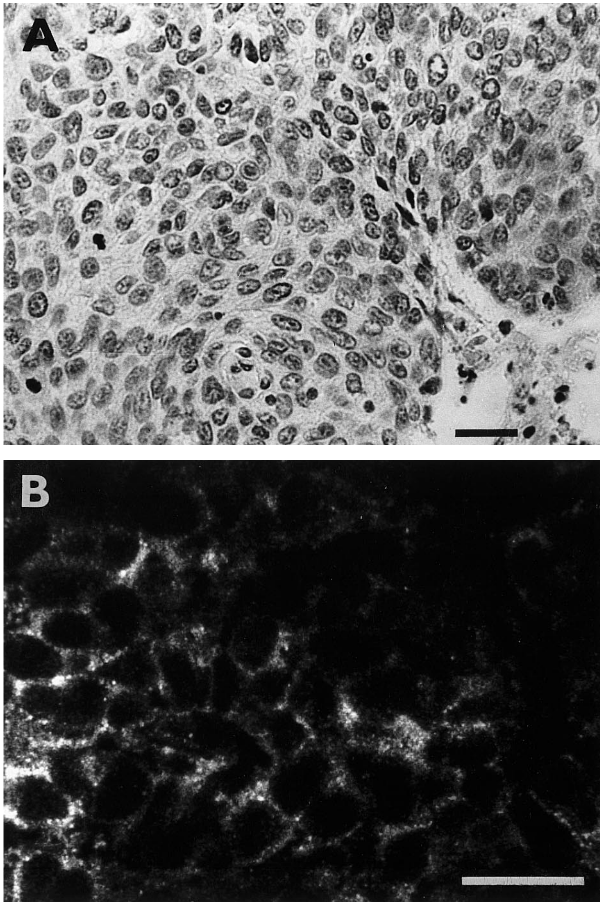

Fig. 7A, B Immunohistochemical staining of squamous cell carcinoma and teratoma with TU-20 antibody. **A** Immunoperoxidase staining of sqamous cell carcinoma of the lungs does not show any immunoreactivity. Nuclei counterstained with haematoxylin. **B** Immunofluorescence staining of a subpopulation of cells in a teratoma. *Bar* 20 μm

ulloblastomas (Fig. 6C). In some neuroblastomas, developing neuronal processes were also stained. In ganglioneuroblastomas and ganglioneuromas staining was observed in perikarya of neuronal cells as well as in their processes. Heterogeneous immunoreactivity in perikarya of neoplastic ganglion cells and strong staining of neuronal processes in ganglioneuroma is shown in Fig. 6D. In the panel of squamous cell carcinomas tested, originating from larynx, lung, mouth floor, pharynx, skin and urinary bladder, the antibody did not give any specific staining (Fig. 7A). Similarly, in other non-neuronal tumours of various tissue origins tested the antibody did not stain neoplastic structures. In surrounding tissues, vascular smooth muscle cells were occasionally very faintly stained. In one case, staining was also found in a teratoma. The stained subpopulation of cells probably represented primitive neural tissue (Fig. 7B). The results of immunostaining on formaldehyde-fixed paraffin-embedded material are summarised in Table 1.

Table 1 Immunohistochemical reactivity of TU-20 with human tumours

Type	Positive/number tested
Ganglioneuroblastoma	3/3
Ganglioneuroma	5/5
Medulloblastoma	2/2
Neuroblastoma	3/3
Sympathoblastoma	1/1
Teratoma	1/3
Adenocarcinoma	0/10
Astrocytoma	0/3
Basalioma	0/3
Glioblastoma	0/3
Hodgkin’s lymphoma	0/2
Leiomyosarcoma	0/3
Melanoma	0/3
Mesothelioma	0/3
Schwanoma	0/2
Squamous cell carcinoma	
Total	0/32
Larynx	0/6
Lungs	0/2
Mouth floor	0/7
Pharynx	0/5
Skin	0/7
Urinary bladder	0/5

Discussion

Differentiation and maturation of neuronal cell types during normal and neoplastic development is accompanied by specific expression of microtubule-associated proteins and tubulins (Molenaar et al. 1989; Lee et al. 1990b). Seven β-tubulin isotype classes are produced in humans, with different tissue distribution (Sullivan 1988). Class III β-tubulin is one of the first cytoskeletal proteins with neuronal specificity to be expressed (Lee et al. 1990a, b) and was detected in a wide variety of neural tissues and tumours of neuroepithelial origin. This isotype was, however, also immunodetected in testis (Ludueña 1993) and in some tumours of non-neuronal origin (Scott et al. 1990). Here we report on the expression of class III β-tubulin detected by a new monoclonal antibody in normal and neoplastic human tissues.

The antibody was prepared against synthetic peptide from the C-terminal end of human class III β-tubulin and its specificity was confirmed by double immunofluorescence with affinity-purified polyclonal antibody against the αβ-tubulin heterodimer, by immunoblotting and by competitive ELISA. The antibody decorated all microtubule structures in mouse neuroblastoma Neuro-2a, and preincubation of the antibody with peptide used for immunisation precluded the labelling (not shown). No granular non-filamentous staining, described for stimulated pheochromocytoma PC-12 cells labelled with polyclonal anti-class III β-tubulin antibody (Asai and Remolona 1989), was observed. In mouse embryonal carcinoma P19 cells, stimulated by retinoic acid, all neuronal processes were continuously stained. No staining was found in other cell lines including PtK_2 and

squamous cell carcinomas lines UM SCC 1, UM SCC 2 and UM SCC 22B. Previously, it was reported that PtK_2 cells (Stearns et al. 1988) and three different squamous cell carcinoma lines were immunostained by antibody against class III β-tubulin (Scott et al. 1990). The results of immunoblotting on carboxymethylated purified tubulin confirmed that the TU-20 antibody did not cross-react either with α-tubulin or with other β-tubulin isotype classes present in brain (Banerjee et al. 1990). Limited reactivity with β-tubulin was also detected on immunoblots after high resolution isoelectric focusing of porcine brain tubulin (Linhartová et al. 1992). The antibody TU-20 stained only subsets of β-tubulin isoelectric variants (isoforms) in comparison with the generic anti-β-tubulin antibody, TU-06. TU-20 stained identical isoforms as antibody SDL.3D10 (Sigma-Aldrich, Prague, Czech Republic), which recognises class III β-tubulin (Banerjee et al.1990; I. Linhartová, P. Dráber, unpublished data). Reactivity with only a minor component of β-tubulin was observed on mouse, rat and porcine whole brain lysates.

Immunohistochemical staining of formaldehyde-fixed paraffin-embedded normal human tissues showed specific staining of cells of neuronal origin in the central and peripheral nervous systems. Faint reactivity was occasionally found in Sertoli cells of the testis. Class III β-tubulin was previously immunodetected in mouse testis by immunoblotting (Frankfurter et al. 1986; Burgoyne et al. 1988; Lewis and Cowan 1988; Lee et al. 1990a) as well as by immunofluorescence in murine Sertoli cells (Lewis and Cowan 1988). In the neoplastic human tissues tested, class III β-tubulin was detected in tumours of neuronal origin and in one case of teratoma, where it was found in a subpopulation of cells. The observed expression of class III β-tubulin detected by antibody TU-20 in human medulloblastoma and neuroblastoma are in accordance with previous immunohistochemical findings in medulloblastoma (Katsetos et al. 1989; Maraziotis et al. 1992), primary explants of medulloblastoma (Vinores et al. 1994) and neuroblastoma (Katsetos et al. 1994). The presence of class III β-tubulin in neuroepithelial components of a spontaneous murine ovarian teratoma was also described (Caccamo et al. 1989). We did not, however, found the isotype in other tumours of non-neuronal origin, including Hodgkin's lymphoma and squamous cell carcinoma. Using the monoclonal antibody TuJ1 against class III β-tubulin, reactivity had previously been reported in B-cell lymphoma and squamous cell carcinoma, but not in normal epithelial cells. It was suggested that the corresponding epitope could serve as a marker for malignant transformation, at least in the case of epithelial cells (Scott et al. 1990).

Observed differences in the staining of squamous cell carcinomas with antibodies TuJ1 and TU-20 are probably not due to the fixation protocols applied, as in both cases formaldehyde-fixed, paraffin-embedded materials were stained. The antibodies most probably recognise different epitopes in the C-terminal region of class III β-tubulin. The TuJ1 antibody was raised against chicken brain tubulin and its epitope is located in the peptide covering the last 14 amino acids on the C-terminal end of chicken class III β-tubulin (Lobert et al. 1995). In this region, the chicken and human class III β-tubulins differ at two amino acid residues (Ludueña 1993). The antigenic determinant recognised by antibody TU-20 could thus specifically masked by posttranslational modification(s) or by binding of microtubule-associated proteins in squamous cell carcinomas. It is well established that C-terminal ends are extensively posttranslationally modified in various β-tubulin isotypes and interact with associated proteins (Ludueña 1993). Alternatively, the sequence of the last eight amino acids on the C-terminal end of human class III β-tubulin, in which the TU-20 epitope is located, is present only in neuronal tissues.

In conclusion, the data presented indicate specific expression of the class III β-tubulin isotype detected by antibody TU-20 only in normal and neoplastic human tissues of neuronal origin. Our findings provide additional evidence that class III β-tubulin is a useful marker for the identification of neuronal neoplasms.

Acknowledgements We thank Dr. M. Nováková for help with immunocompetitive tests and Dr. A. Banerjee (University of Texas, San Antonio, USA) and Dr. A. Smertenko (University of London, Egham, UK) for help with carboxymethylation of tubulin and separation of class III β-tubulin. Cell lines UM SCC 1, UM SCC 2 and UM SCC 22B were kindly provided by Dr. T.E. Carey (University of Michigan, Ann Arbor, USA). This work was supported in parts by grant 301/94/1674 from GA ČR and by EC grant ERB 3510 PL927710.

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