PERGAMON

European Journal of Cancer 37 (2001) 114–121

European Journal of Cancer

www.ejconline.com

Heterogeneous lack of expression of the tumour suppressor PTEN protein in human neoplastic tissues

J. Torres [a,†], S. Navarro [b,†], I. Roglá [a], F. Ripoll [a], A. Lluch [c], J. García-Conde [c], A. Llombart-Bosch [b], J. Cervera [a], R. Pulido [a,*]

[a] *Instituto de Investigaciones Citológicas, Amadeo de Saboya 4, 46010 Valencia, Spain*
[b] *Departamento de Patología, Universidad de Valencia, Spain*
[c] *Servicio de Oncología y Hematología, Hospital Clínico de Valencia, Universidad de Valencia, Spain*

Received 8 February 2000; received in revised form 25 July 2000; accepted 26 July 2000

Abstract

PTEN, a tumour suppressor gene located at chromosome 10q23 and commonly mutated or deleted in a variety of tumours, encodes a dual-specific/phosphatidylinositol-3,4,5-triphosphate (PIP3) phosphatase. We report the generation of an anti-PTEN monoclonal antibody (MAb) that recognises an epitope at the C-terminus of PTEN, and describe the heterogeneous lack of expression of the PTEN protein in human tumour tissues, as demonstrated by immunohistochemical methods. Our anti-PTEN MAb provides a useful tool for the study of PTEN protein expression in tumour samples, in the search for tumour prognostic molecular markers. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *PTEN*; Tumour suppressor; Cancer; Protein phosphatases

1. Introduction

Alterations in the expression and/or the function of proteins encoded by oncogenes and tumour suppressor genes are key factors in the triggering of cell transformation and tumorigenicity. The tumour suppressor gene *PTEN/MMAC1/TEP-1* (hereafter, *PTEN*) is deleted or mutated in multiple human cancers, including breast, prostate, endometrial and lung carcinomas, glioblastomas, and melanomas [1–3]. In addition, germ line mutations in *PTEN* have been found in inherited cancer syndromes, such as Cowden disease, Lhermitte–Duclos disease and Bannayan–Zonana syndrome; these diseases share clinical manifestations that include the presence of multiple benign tumours and hamartomas, as well as abnormal organ development, mainly in the central nervous system [4–6]. *PTEN* encodes an enzyme with phosphatase activity towards both acidic protein substrates and the lipid second messenger, phosphatidylinositol-3,4,5-triphosphate (PIP3) [7,8]. The PIP3 phosphatase activity of PTEN is crucial to control the phosphatidylinositol-3 (PI-3) kinase signal transduction pathway and the activation of the protein kinase B *(PKB)/Akt* proto-oncogene [9–17]. PTEN also regulates cell motility and invasion by dephosphorylation of the focal adhesion kinase (FAK) and the adapter protein Shc [18–21]. Finally, mice with targeted homozygous *PTEN* deletions show early embryonic lethality, as a consequence of defects in embryonic cell differentiation and high susceptibility to cancer in multiple organs [22–24]. Interestingly, *PTEN*+/− heterozygous mice show severe neoplasia and autoimmunity processes due to defects in apoptosis [25,26].

Differences in the incidence of *PTEN* mutations have been found in distinct types of sporadic tumours, as well as in tumours at distinct stages of disease. Thus, glioblastoma multiforme, malignant melanomas, and advanced prostate carcinomas show an incidence of *PTEN* mutation of 20–60% [27–29], whereas lower mutation percentages are found in low-grade gliomas or primary breast carcinomas [30–32]. These studies have focused on the analysis of *PTEN* gene sequence, both at its coding region and at the exon–intron boundaries. Thus, the regulation of the expression of PTEN at the protein level, and its relationship with tumour progres-

* Corresponding author. Tel.: +34-96-3391256; fax: +34-96-3601453.

† J. Torres and S. Navarro contributed equally to this work.

E-mail address: rpulido@ochoa.fib.es (R. Pulido).

PII: S0959-8049(00)00366-X

sion, is poorly understood. Interestingly, recent reports have shown lack of expression of the PTEN protein in distinct tumour samples [33–35]. To further study the expression of the PTEN protein, we have generated an anti-PTEN monoclonal antibody (MAb) (named 17.A) which recognises an epitope located at the C-terminus of this molecule. This was used to study immunohistochemically the expression of PTEN in several neoplasms.

2. Materials and methods

2.1. Plasmid construction and purification of recombinant proteins

The cDNA encoding full-length human PTEN was obtained by reverse transcriptase-polymerase chain reaction (RT-PCR) amplification of mRNA from MCF-7 cells, using primers flanking the human *PTEN* coding region [1–3], the sequence being confirmed by DNA sequencing. MCF-7 poly-A+ mRNA was isolated using the Quick Prep Micro mRNA purification kit (Amersham Pharmacia Biotech.) pRK5-PTEN and pRK5-glutathione-S-transferase (GST)-PTEN (full length and truncated forms) mammalian expression vectors were made by PCR amplification of *PTEN* or *GST* (*S. japonicum* sequence) cDNAs and subcloning. For pRK5-PTEN, a 5′ primer containing a Kozak sequence was used for the initiation of translation. For the construction of bacteria expression plasmids encoding GST-fusion proteins, full length PTEN, or truncated forms, were subcloned into the expression vector pGEX-4T2 (GST-PTEN 1-403, and GST-PTEN 1-202) or pGEX-5X1 (GST-PTEN 203-403). Purification of GST-fusion proteins was done by standard procedures, using Glutathione-Sepharose beads (Amersham Pharmacia Biotech.). To obtain recombinant purified PTEN, GST-PTEN was digested for 4 h at room temperature with 15 units/mg of thrombin (Sigma Chemical Co.), followed by the removal of GST by incubation with Glutathione-Sepharose beads.

2.2. Generation of antibodies

For the production of anti-PTEN MAb-secreting hybridomas, Balb/c mice were immunised with recombinant PTEN following standard procedures, and spleen cells were fused with myeloma SP-20 cells. Screening of MAb-secreting clones was carried out by an enzyme-linked immunosorbent assay (ELISA), using recombinant PTEN bound to plastic as the antigen. Briefly, 96-well plates were coated with GST–PTEN (100 μl, 1 μg/ml) for 16 h at 4°C. After blocking, 50 μl of culture supernatants were added and incubated for 2 h at room temperature, followed by washing and incubation for 2 h with the peroxidase-conjugated secondary antibody. After washing, 100 μl of developing solution (25 mM citrate, 50.4 mM phosphate buffer, pH 5.0, containing 1 mg/ml o-phenylenediamine, 0.325 μl H_2O_2 (30%)/ml) were added, and the absorbance was read at 492 nm. Dilution of antigens and antibodies was made in blocking solution (phosphate-buffered solution (PBS), 1% bovine serum albumin (BSA), 0.1% non-fat milk). 17.A clone (IgM,κ) was selected and subjected to further cloning. The rabbit polyclonal anti-PTEN 3′ was obtained by rabbit immunisation with GST-PTEN 203–403. The rabbit polyclonal anti-PTEN CS486 antibody was provided by R. Parsons [17]. The 22.A anti-GST MAb was obtained in our laboratory, and will be described elsewhere (data not shown). Horseradish peroxidase-conjugated goat anti-rabbit and anti-mouse secondary antibodies were from Oncogene Research Products and Promega Corporation.

2.3. Cell culture, transfections, immunoprecipitation and immunoblot

Human embryonic kidney 293 and simian COS-7 cells were grown in Dulbecco's minimal essential medium (DMEM) containing high glucose (4.5 g/l) supplemented with 5% (for COS-7 cells) or 10% heat-inactivated fetal calf serum (FCS) (Life Technologies, Inc.). Human mammary carcinoma MCF-7 cells were grown in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% heat-inactivated (FCS). COS-7 and 293 cells were transfected by the diethylaminoethanol (DEAE)-dextran or the calcium phosphate precipitation methods, respectively, and processed 48–72 h after transfection. For [^{35}S]methionine labelling, cells were cultured for 4 h with L-methionine-free DMEM and 2% dialysed FCS in the presence of [^{35}S]methionine (50 μCi/ml). Cell lysis, immunoprecipitations and immunoblot were performed as described in Ref. [36]. For immunoprecipitations with the 17.A MAb, a goat anti-mouse Ig was used as secondary antibody, followed by protein A-Sepharose.

2.4. Immunoperoxidase staining of tissue sections

Immunohistochemistry was performed as previously described in Ref. [37]. Briefly, tumour tissue samples, obtained by standard surgical procedures, were fixed in buffered 10% formaldehyde and embedded in paraffin. Five μm-sections were quenched for 15 min in 3% hydrogen peroxide in methanol, washed with PBS, and incubated with 20% horse serum for 20 min. Then, sections were incubated with 17.A MAb (culture supernatant; 10 μg/ml approximately) for 45 min at room temperature. The time of incubation for the secondary antibody and avidin–biotin complexes was 30 min at room temperature. Samples were washed and developed

using 0.05% 3,3′-diamino-benzidine, tetrachloride in 0.1% hydrogen peroxide. As a control of specificity, incubation of the samples in the presence of an excess of recombinant PTEN blocked the reactivity of the 17.A MAb. As negative controls, 17.A was substituted for a non-reactive Ig from mouse ascites. Immunostaining of human normal tissues was performed on a multi-tissue control block (Biomeda Corp.); tissue sections stained included thyroid, spleen, uterus, placenta, prostate, tonsil, testis, ovary, pancreas, lung, liver, fetal liver, skin, kidney, breast, heart, stomach, small intestine, large intestine, brain, pituitary and adrenal gland. All normal tissue sections were positive for the 17.A MAb.

3. Results

To study the expression of the tumour suppressor PTEN protein, a hybridoma clone (17.A) secreting an anti-PTEN monoclonal antibody (MAb) was generated. The 17.A anti-PTEN MAb reacted by ELISA with purified recombinant GST–PTEN 1–403 (spanning the full length human PTEN sequence) and GST–PTEN 203–403 (human PTEN C-terminal portion) fusion proteins, but not with GST alone or GST-PTEN 1–202 (human PTEN N-terminal portion) (Fig. 1a). Furthermore, the 17.A MAb reacted by immunoblot with the GST–PTEN 1–403 fusion protein overexpressed in 293 cells, but not with GST–PTEN 1–369 or 1–386 C-terminus truncated forms (Fig. 1b). Thus, the epitope recognised by the 17.A MAb is located in the C-terminal portion of PTEN, probably between amino acids 386–403. In addition, the 17.A MAb recognised recombinant untagged PTEN, overexpressed in COS-7 or 293 cells, by immunoprecipitation or immunoblot (Fig. 1c and d, respectively). As controls, the recognition of the same recombinant PTEN molecules with two distinct anti-PTEN rabbit polyclonal antisera is shown (Fig. 1c, lane 3; and d, lane 4).

The 17.A MAb was used to study the expression of PTEN in different human tissues by immunohistochemical techniques. 17.A immunostaining was detected in all human normal tissues analysed (NormalGrid^{TM}:Multi-tissue Control Slides (Fig. 2a–d; data not shown)), indicating that PTEN protein is widely expressed, as expected from the reported wide expression of *PTEN* mRNA [2]. In general, the pattern of immunostaining in tissue sections was mainly diffuse in the cell cytoplasm, with weak nuclear impregnation. Next, PTEN expression was investigated in a panel of paraffin-embedded human tumour tissue sections. A high number of tumour samples, including distinct types of carcinomas, lymphomas, sarcomas and neuroblastomas, were found to be negative for PTEN expression (Table 1). Control, normal tissues adjacent to the tumour areas (when present) were positively stained. Samples were also found expressing PTEN to a variable extent, as indicated by 17.A reactivity (Table 1). Some seminoma and melanoma samples were also negative for 17.A staining (Table 1), although from the small number of samples analysed for these types of tumours, any further conclusions cannot be drawn. An example of the heterogeneous staining found with the 17.A MAb is illustrated in Fig. 2(e–h), for ductal breast tumour samples. As shown, 17.A-positive and -negative breast carcinomas were found (Fig. 2e and f, respectively). Some tumours showed the existence of clusters of 17.A-positive cells together with 17.A-negative cells (Fig. 2g), revealing a heterogeneity in the neoplastic cells within the tumour. In some cases, a defined nuclear staining by 17.A was observed; whereas in others, the pattern of staining was diffuse in both the cytoplasm and nucleus (data not shown). Finally, metastatic cells from a breast tumour were found to be completely negative for 17.A staining

Table 1
PTEN protein expression in human neoplasms

Tumours	Number of cases *n*	ø *n* (%)	+ *n* (%)	+ + *n* (%)	+ + + *n* (%)
Invasive ductal breast carcinomas	32	16 (50)	12 (38)	2 (6)	2 (6)
Lymphomas	20	14 (70)	5 (25)	1 (5)	0
High grade soft tissue spindle cell sarcomas	14	7 (50)	5 (36)	2 (14)	0
Neuroblastomas	9	6 (67)	2 (22)	0	1 (11)
Colon adenocarcinomas	5	0	0	3 (60)	2 (40)
Squamous lung carcinomas	4	1 (25)	3 (75)	0	0
Renal cell carcinomas	4	0	2 (50)	1 (25)	1 (25)
Hepatocarcinomas	3	3 (100)	0	0	0
Osteosarcomas	2	1 (50)	1 (50)	0	0
Ewing's sarcomas	2	2 (100)	0	0	0
Carcinoids of the appendix	2	1 (50)	1 (50)	0	0
Seminomas	2	2 (100)	0	0	0
Melanomas	3	1 (33)	2 (67)	0	0

Score: ø, 0–10% positivity; +, 10–30%; + +, 30–70%; + + +, 70–100%.

(Fig. 2h), suggesting that an inverse correlation between PTEN protein expression and malignancy could exist.

4. Discussion

The gene encoding the tumour suppressor phosphatase PTEN is deleted or mutated in a wide number of tumours, including glioblastomas, diverse carcinomas, and melanomas [1,2]. In this report, we describe the generation of an anti-PTEN MAb (17.A) suitable to study the expression of PTEN in tumour tissues. Endogenous PTEN from normal tissue sections, as well as from a variable number of tumour specimens, was strongly stained by immunohistochemistry using the 17.A MAb (see Fig. 2). The low sensitivity, using either immunoblotting or immunoprecipitation, of the 17.A MAb towards PTEN should be noted. This hampered

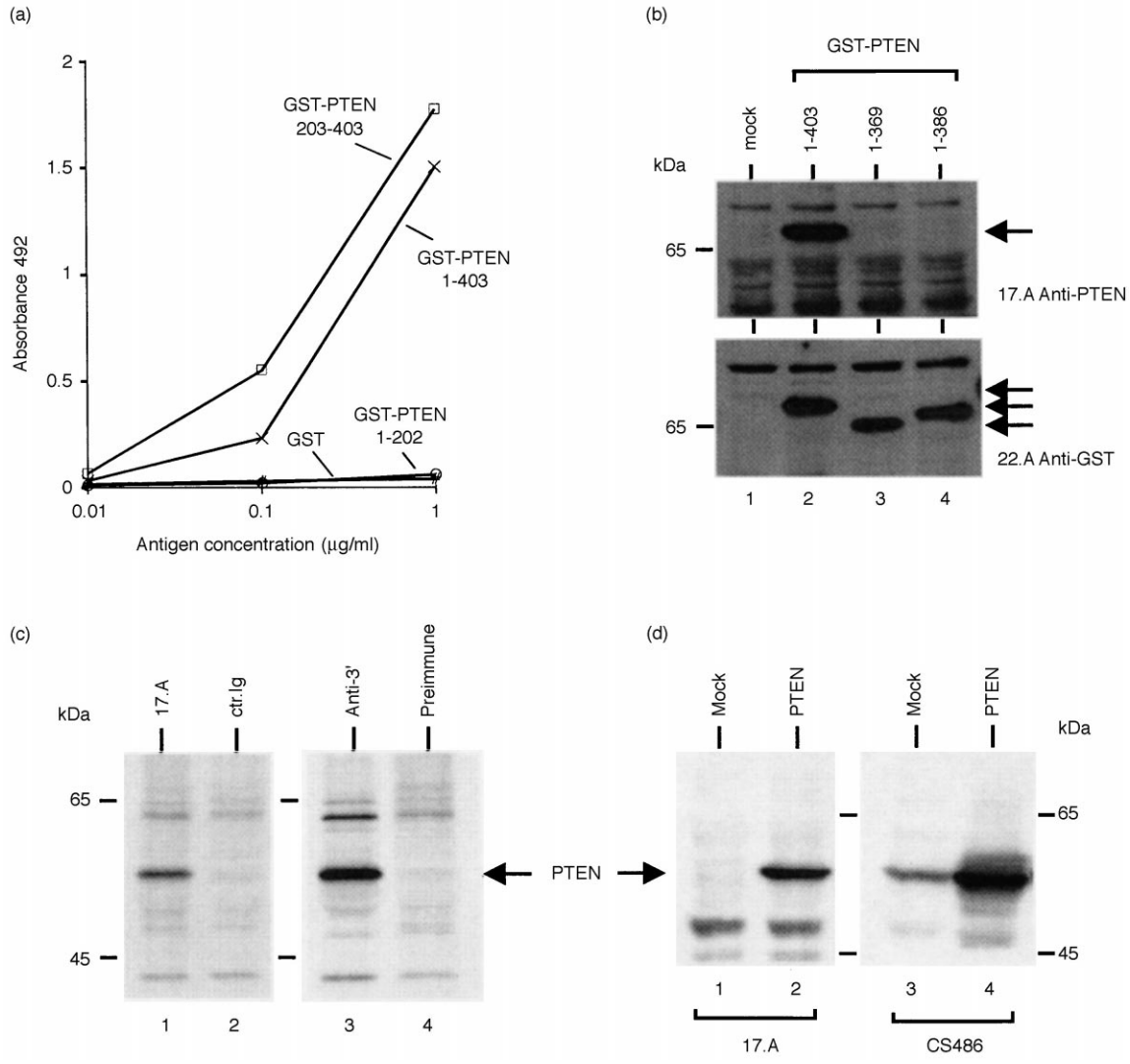

Fig. 1. 17.A monoclonal antibody (MAb) recognises the C-terminus of human PTEN. (a) 17.A culture supernatant (50 μl) was added to 96-well plates coated with glutathione-S-transferase (GST) or GST–PTEN fusion proteins, as indicated, and reactivity was measured by indirect enzyme-linked immunosorbent assay (ELISA), using goat anti-mouse peroxidase as the secondary antibody. (b) 293 cells were mock-transfected (pRK5 vector alone; lane 1) or transfected with pRK5–GST–PTEN full-length (1–403) (lane 2) or pRK5–GST–PTEN truncated forms (1–369 and 1–386) (lanes 3 and 4), as indicated. Postnuclear cell lysates (50 μg) were subjected to immunoblotting with the 17.A anti-PTEN (upper panel) or the 22.A anti-GST MAb (lower panel). Arrows in the right indicate the migration of the distinct GST-PTEN fusion proteins. (c) COS-7 cells were transfected with pRK5-PTEN and labelled with [^{35}S]methionine, followed by immunoprecipitation with 17.A (lane 1), control immunoglobulin (ctr.Ig) (lane 2), anti-PTEN 3′ (anti-3′) rabbit antiserum (lane 3), or preimmune serum (lane 4). (d) 293 cells were transfected with pRK5 vector alone (mock) or pRK5-PTEN, and postnuclear cell lysates (50 μg) were subjected to immunoblotting using 17.A MAb (lanes 1–2) or CS486 anti-PTEN rabbit antiserum (lanes 3–4). In (b), (c) and (d), samples were resolved by 10% sodium dodecyl sulphate–polyacrylamide gel (SDS–PAGE) under reducing conditions. Note that (b) shows expression of recombinant truncated or full-length GST–PTEN (70–85 kDa), whereas (c) and (d) show expression of endogenous or recombinant untagged PTEN (55 kDa).

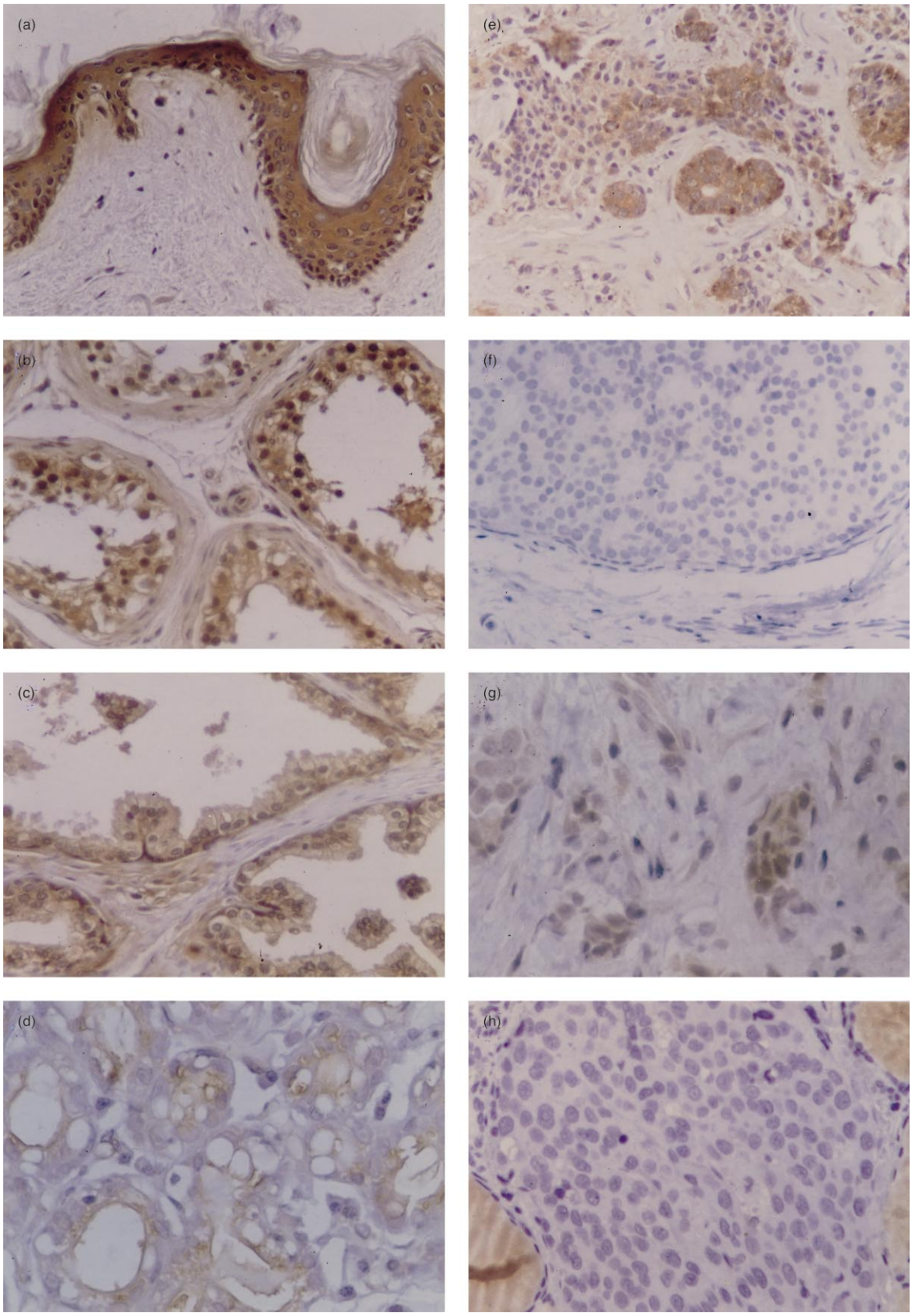

Fig. 2. Immunoperoxidase staining with 17.A anti-PTEN MAb of human normal (a–d) and invasive ductal breast carcinoma (e–h) tissue samples. (a) Skin; (b) testis; (c) prostate; (d) lactating breast; (e) a breast tumour that is 17.A-positive; (f) a breast tumour that is 17.A-negative; (g) a breast tumour that is 17.A-partially negative, showing the presence of a cluster of 17.A-positive cells; (h) breast tumour metastases in the thyroid gland that is 17.A-negative.

the detection of the endogenous molecule by these techniques (compare the reactivity of 17.A with that shown by the two anti-PTEN antisera; Fig. 1c, lane 1 versus lane 3; and 1d, lane 2 versus lane 4). Interestingly, a variable pattern of intracellular staining by the anti-PTEN 17.A MAb was observed in tissue sections. In addition, the subcellular distribution of PTEN was found to be different when immunofluorescence staining on transfected COS-7 and 293 cells was compared. Thus, recombinant PTEN was mainly cytosolic in COS-7 cells, whereas in 293 cells recombinant PTEN was present both in the nucleus and in the cytosol (data not shown). Whether the PTEN protein possesses a cell type-specific subcellular location, or PTEN overexpression in some cell lines bypasses a putative mechanism of nuclear targeting, deserves further analysis. In this regard, both nuclear and cytoplasmic locations have been described for PTEN depending upon the cell types [3,38–40], and a differential nuclear and cytoplasmic PTEN expression has been reported for normal and tumoral thyroid tissues [41].

Alterations of the *PTEN* gene have been found more frequently in late-stage tumours, such as glioblastoma multiforme, advanced prostate cancer or malignant melanomas [27,28,30,42]. Interestingly, we have observed on some tumour sections, the existence of 17.A-positive and -negative cells within the same tumour area, suggesting the co-existence of cells at distinct malignant stages in such tumours. In addition, the possibility exists that there is a cycle of PTEN expression throughout the tumour cells that is out of synchrony. These findings support the notion that expression of PTEN in tumour biopsies, as analysed in this study by using our 17.A anti-PTEN MAb, could be used as a prognostic factor for tumour aggression. A larger screening of tumour samples, in correlation with the clinical tumour metastatic and aggressiveness parameters, will be necessary before further conclusions can be drawn. In this context, altered PTEN expression has been postulated as a diagnostic marker for premalignant endometrial cancers [43].

We have found a high number of 17.A-negative tumours in the randomly selected samples analysed, in comparison with the available statistics for *PTEN* gene alterations in the equivalent tumours. For instance, *PTEN* mutations in breast tumours or lymphomas have been found with a 4–5% frequency [44], whereas a much higher percentage of the breast carcinomas (16 out of 32) of the lymphoma samples (14 out of 20) analysed in this study were negative for 17.A staining. Furthermore, a high frequency of PTEN protein loss of expression has also been found in glioblastoma multiforme, carcinoma of prostate and primary ductal breast adenocarcinoma samples [33–35]. Several causes could account for the high frequency of tumour samples lacking PTEN protein expression, including undetected gene alterations in the exon–intron boundaries or in the promoter region of the *PTEN* gene, as well as secondary gene alterations resulting in the loss of protein expression. Since the 17.A anti-PTEN MAb recognises a C-terminal epitope on PTEN, the possibility exists that PTEN truncated molecules could be expressed in some of the tumours that were negative for the 17.A MAb. For example, a 2-bp deletion in the *PTEN* gene resulting in a stop codon at amino acid position 385 has been found in a primary breast tumour [31], suggesting that the C-terminal portion of PTEN could be important for its tumour suppressor function. Thus, the PTEN C-terminal domain has been proposed to target PTEN to membrane; and mutations within this domain ablate both PTEN membranes binding and its tumour suppressor function [45,46]. In addition, the C-terminal tail of PTEN confers protein stability, likely by its association with scaffolding molecules [47]. Further work will be necessary to elucidate the role of PTEN protein stability in the context of suppression of cell transformation. The use of anti-PTEN antibodies directed against well-defined epitopes in distinct parts of the molecule will facilitate such analyses.

Acknowledgements

This work was supported by grants from Generalitat Valenciana (GV-C-VS-20-047-96) and from Fondo de Investigaciones Sanitarias (FIS 99/1197) (to S. Navarro), Spain. J. Torres was supported by fellowships from the Fundación Científica de la Asociación Española contra el Cáncer and from Instituto de Investigaciones Citológicas. We thank R. Parsons and C. Eng for providing antibodies; M.C. Sanfeliu, C. Clock and L. Couchonnal for their valuable assistance; and M.E. Armengod for critical reading of the manuscript.

References

1. Li J, Yen C, Liaw D, *et al*. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 1997, **275**, 1943–1947.
2. Steck PA, Pershouse MA, Jaser SA, *et al*. Identification of a candidate tumour suppressor gene, *MMAC1*, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nature Genet* 1997, **15**, 356–362.
3. Li D-M, Sun H. TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor β. *Cancer Res* 1997, **57**, 2124–2129.
4. Liaw D, Marsh DJ, Li J, *et al*. Germline mutations of the PTEN gene in Cowden disease, and inherited breast and thyroid cancer syndrome. *Nature Genet* 1997, **16**, 64–67.
5. Marsh DJ, Dahia PLM, Zheng Z, *et al*. Germline mutations in PTEN are present in Bannayan-Zonana syndrome. *Nature Genet* 1997, **16**, 333–334.
6. Nelen MR, van Staveren WCG, Peeters EAJ, *et al*. Germline mutations in the PTEN/MMAC1 gene in patients with Cowden disease. *Hum Mol Genet* 1997, **6**, 1383–1387.

7. Myers MP, Stolarov JP, Eng C, *et al*. P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. *Proc Natl Acad Sci USA* 1997, **94**, 9052–9057.
8. Maehama T, Dixon JE. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-triphosphate. *J Biol Chem* 1998, **273**, 13375–13378.
9. Cantley LC, Neel BG. New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc Natl Acad Sci USA* 1999, **96**, 4240–4245.
10. Maehama T, Dixon JE. PTEN: a tumour suppressor that functions as a phospholipid phosphatase. *Trends Cell Biol* 1999, **9**, 125–128.
11. Haas-Kogan D, Shalev N, Wong M, Mills G, Yount G, Stokoe D. Protein kinase B (PKB/Akt) activity is elevated in glioblastoma cells due to mutation of the tumor suppressor *PTEN/MMAC1*. *Curr Biol* 1998, **8**, 1195–1198.
12. Myers MP, Pass I, Batty IH, *et al*. The lipid phosphatase activity of PTEN is critical for its tumor supressor function. *Proc Natl Acad Sci USA* 1998, **95**, 13513–13518.
13. Wu X, Senechal K, Neshat MS, Whang YE, Sawyers CL. The PTEN/MMAC1 tumor suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase/Akt pathway. *Proc Natl Acad Sci USA* 1998, **95**, 15587–15591.
14. Dahia PLM, Aguiar RCT, Alberta J, *et al*. *PTEN* is inversely correlated with the cell survival factor Akt/PKB and is inactivated via multiple mechanisms in haematological malignancies. *Human Mol Genet* 1999, **8**, 185–193.
15. Davies MA, Lu Y, Sano T, *et al*. Adenoviral transgene expression of *MMAC/PTEN* in human glioma cells inhibits Akt activation and induces anoikis. *Cancer Res* 1998, **58**, 5285–5290.
16. Li D-M, Sun H. PTEN/MMAC1/TEP1 suppresses the tumorigenicity and induces G1 cell cyle arrest in human glioblastoma cells. *Proc Natl Acad Sci USA* 1998, **95**, 15406–15411.
17. Li J, Simpson L, Takahashi M, *et al*. The *PTEN/MMAC1* tumor suppressor induces cell death that is rescued by the AKT/protein kinase B oncogene. *Cancer Res* 1998, **58**, 5667–55672.
18. Tamura M, Gu J, Matsumoto K, Aota S, Parsons R, Yamada KM. Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. *Science* 1998, **280**, 1614–1617.
19. Tamura M, Gu J, Takino T, Yamada KM. Tumor suppressor PTEN inhibition of cell invasion, migration, and growth: differential involvement of focal adhesion kinase and p130Cas. *Cancer Res* 1999, **59**, 442–449.
20. Gu J, Tamura M, Yamada KM. Tumor suppressor PTEN inhibits integrin- and growth factor-mediated mitogen-activated protein (MAP) kinase signaling pathways. *J Cell Biol* 1998, **143**, 1375–1383.
21. Gu J, Tamura M, Pankov R, *et al*. Shc and FAK differentially regulate cell motility and directionality modulated by PTEN. *J Cell Biol* 1999, **146**, 389–403.
22. Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP. *Pten* is essential for embryonic development and tumour suppression. *Nature Genet* 1998, **19**, 348–355.
23. Stambolic V, Suzuki A, de la Pompa JL, *et al*. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell* 1998, **95**, 29–39.
24. Suzuki A, de la Pompa JL, Stambolic V, *et al*. High cancer susceptibility and embryonic lethality associated with mutation of the *PTEN* tumor suppressor gene in mice. *Curr Biol* 1998, **8**, 1169–1178.
25. Podsypanina K, Ellenson LH, Nemes A, *et al*. Mutation of Pten/Mmac1 in mice causes neoplasia in multiple organ systems. *Proc Natl Acad Sci USA* 1999, **96**, 1563–1568.
26. Di Cristofano A, Kotsi P, Peng YF, Cordon-Cardo C, Elkon KB, Pandolfi P. Impaired Fas response and autoimmunity in Pten +/− mice. *Science* 1999, **285**, 2122–2125.
27. Guldberg P, thor Straten P, Birck A, Ahrenkiel V, Kirkin AF, Zeuthen J. Disruption of the *MMAC1/PTEN* gene by deletion or mutation is a frequent event in malignant melanoma. *Cancer Res* 1997, **57**, 3660–3663.
28. Wang SI, Puc J, Li J, *et al*. Somatic mutations of PTEN in glioblastoma multiforme. *Cancer Res* 1997, **57**, 4183–4186.
29. Chiariello E, Roz L, Albarosa R, Magnani I, Finocchiaro G. *PTEN/MMAC1* mutations in primary glioblastomas and short-term cultures of malignant gliomas. *Oncogene* 1998, **16**, 541–545.
30. Rasheed BKA, Stenzel TT, McLendon RE, *et al*. PTEN gene mutations are seen in high-grade but not in low-grade gliomas. *Cancer Res* 1997, **57**, 4187–4190.
31. Rhei E, Kang L, Bogomolniy F, Federici MG, Borgen PI, Boyd J. Mutation analysis of the putative tumor suppressor gene *PTEN/MMAC1* in primary breast carcinomas. *Cancer Res* 1997, **57**, 3657–3659.
32. Somerville RPT, Shoshan Y, Eng C, Barnett G, Miller D, Cowell JK. Molecular analysis of two putative tumour suppressor genes, *PTEN* and *DMBT*, which have been implicated in glioblastoma multiforme disease progression. *Oncogene* 1998, **17**, 1755–1757.
33. Sano T, Lin H, Chen X, *et al*. Differential expression of *MMAC/PTEN* in glioblastoma multiforme: relationship to localization and prognosis. *Cancer Res* 1999, **5**, 1820–1824.
34. McMenagin ME, Soung P, Perera S, Kaplan I, Loda M, Sellers WR. Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. *Cancer Res* 1999, **59**, 4291–4296.
35. Perren A, Weng L-P, Boag AH, *et al*. Immunohistochemical evidence of loss of PTEN expression in primary ductal adenocarcinomas of the breast. *Am J Pathol* 1999, **155**, 1253–1260.
36. Pulido R, Zúñiga Á, Ullrich A. PTP–SL and STEP protein tyrosine phosphatases regulate the activation of the extracellular signal-regulated kinases ERK1 and ERK2 by association through a kinase interaction motif. *EMBO J* 1998, **17**, 7337–7350.
37. Navarro S, Colamonici OR, Llombart-Bosch A. Immunohistochemical detection of the type I interferon receptor in human fetal, adult, and neoplastic tissues. *Mod Pathol* 1996, **9**, 150–156.
38. Furnari FB, Lin H, Su Huang H-J, Cavenee WK. Growth suppression of glioma cells by PTEN requires a functional phosphatase catalytic domain. *Proc Natl Acad Sci USA* 1997, **94**, 12479–12484.
39. Lachyankar MB, Sultana N, Schonhoff CM, *et al*. A role for nuclear PTEN in neuronal differentiation. *J Neurosci* 2000, **20**, 1404–1413.
40. Hlobilkova A, Guldberg P, Thullberg M, Zeuthen J, Lukas J, Bartek J. Cell cycle arrest by the PTEN tumor suppressor is target cell specific and may require protein phosphatase activity. *Exp Cell Res* 2000, **256**, 571–577.
41. Gimm O, Perren A, Weng L-P, *et al*. Differential nuclear and cytoplasmic expression of PTEN in normal thyroid tissue, and benign and malignant epithelial thyroid tumors. *Am J Pathol* 2000, **156**, 1693–1700.
42. Whang YE, Wu X, Suzuki H, *et al*. Inactivation of the tumor suppressor PTEN/MMAC1 in advanced human prostate cancer through loss of expression. *Proc Natl Acad Sci USA* 1998, **95**, 5246–5250.
43. Mutter GL, Lin M-C, Fitzgerald JT, *et al*. Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. *J Natl Cancer Inst* 2000, **92**, 924–931.
44. Ali IU, Schriml LM, Dean M. Mutational spectra of PTEN/MMAC1 gene: a tumor suppressor with lipid phosphatase activity. *J Natl Cancer Inst* 1999, **91**, 1922–1932.
45. Lee J-O, Yang H, Georgescu M-M, *et al*. Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association. *Cell* 1999, **99**, 323–334.

46. Georgescu M-M, Kirsch KH, Akagi T, Shishido T, Hanafusa H. The tumor-suppressor activity of PTEN is regulated by its carboxyl-terminal region. *Proc Natl Acad Sci USA* 1999, **6**, 10182–10187.

47. Wu X, Hepner K, Castelino-Prabhu S, *et al*. Evidence for regulation of the PTEN tumor suppressor by a membrane-localized multi-PDZ domain containing scaffold protein MAGI-2. *Proc Natl Acad Sci USA* 2000, **97**, 4233–4238.