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Differential Expression of the PTEN Tumor Suppressor Protein in Fetal and Adult Neuroendocrine Tissues and Tumors: Progressive Loss of PTEN Expression in Poorly Differentiated Neuroendocrine Neoplasms

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Genetic alteration and loss of expression of tumor suppressor gene PTEN has been found in carcinomas of the breast, prostate, and endometrium, as well as in gliomas. PTEN expression in neural crest/neuroendocrine (NC/NE) tissues and in neoplasms has not been reported. This study examines PTEN expression in embryonal, fetal, and adult tissues by immunohistochemistry. The authors found high PTEN expression in embryonal, fetal, and adult NC/NE tissues. The authors also study the PTEN expression in NC/NE neoplasms (N = 37), including 5 melanocytic nevi, 2 melanomas, 9 carcinoids, 2 moderately differentiated neuroendocrine carcinomas, 13 poorly differentiated neuroendocrine carcinomas, 2 paragangliomas, 2 pheochromocytomas, 2 medullary thyroid carcinomas, and 1 neuroblastoma. All carcinoid tumors and melanocytic nevi showed moderate or strong immunostaining for PTEN. In contrast, the majority of poorly differentiated neuroendocrine carcinomas (7 of 13) were negative for PTEN (54%); the remainder showed diminished reactivity. The two melanomas studied were also negative for PTEN immunostaining. The paragangliomas, pheochromocytomas, medullary thyroid carcinomas, and neuroblastoma all showed a strong PTEN stain. The authors postulate that PTEN is a differentiation marker for NC/NE tissue and tumors and that loss of PTEN expression may represent an important step in the progression of NE tumors.

Key Words: PTEN-tumor suppressor gene—Immunohistochemistry—Neuroendocrine—Carcinoid—Neuroendocrine carcinoma.

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PTEN is a recently identified tumor suppressor gene mapped to chromosome TEN, having both lipid/tyrosine phosphatase activity and molecular homology to tensin (1,2). It is thought to play an important role in the fundamental control of cell growth, death, adhesion, and migration (3–6). Transgenic mice, which harbor disrupted PTEN, manifest early embryonic lethality, whereas heterologous mice that carry the PTEN+/- gene spontaneously develop tumors in variety of organs, suggesting a dual role in ontogenesis and tumor suppression (7). Germline mutations of PTEN have been detected in three human autosomal dominant disorders, Cowden disease, L'Hermitte-Duclos disease, and Bannayan-Zonana syndrome, which share some similar features including the formation of multiple benign tumors and an increased susceptibility to malignancy development (8–12). A high incidence of PTEN mutations has also been observed in several tumors, including glioma, endometrial, and breast carcinomas (13–19). Furthermore, loss of PTEN protein expression is frequent in high-grade breast and prostate carcinomas, suggesting that the loss of PTEN is a late event in the progression of these tumors (20–22).

Recently, Gimm et al.(23) found high PTEN expression in fetal and adult central and peripheral nervous systems. These data prompted us to investigate, using immunohistochemical methods, PTEN protein expression in fetal and adult neural crest/neuroendocrine (NC/NE) tissues. We observed that PTEN protein was expressed in the fetal and adult pituitary glands, pancreatic islets, adrenal medulla, peripheral nerve plexuses, and skin melanocytes. We subsequently investigated PTEN expression in a variety of NE neoplasms and found that progressive loss of PTEN protein expression occurred in poorly differentiated tumors.

MATERIALS AND METHODS

Tissues and Tumors

The material studied comprised tissues from six embryos and fetuses (gestational age: 8, 11, 13, 17, 22, and 25 weeks), two adults, and 37 NC/NE neoplasms (Table 1) obtained from archival surgical and autopsy specimens in the Department of Pathology, Kings County Hospital Center, Brooklyn, New York. Blocks fixed in 10% buffered formalin and embedded in paraffin were evaluated independently (by C.A.A. and L.W.) for gestational age, tissue type, and diagnosis. Immunostaining

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TABLE 1. *Grade and semiquantitative scoring of PTEN immunostain in NC tumors*

Case	Diagnosis	Staining intensity	Positive cells (%)
1	Carcinoid, thymus	+++	++++
2	Carcinoid, teratoma	++	++++
3	Carcinoid, colon	+++	++++
4	Carcinoid, colon	+++	++++
5	Carcinoid, colon	+++	++++
6	Carcinoid, liver	+++	++++
7	Carcinoid, mediastinum	++	++++
8	Carcinoid, appendix	+++	++++
9	Carcinoid, small intestine	++	++++
10	MDNEC, kidney	++	+++
11	MDNEC, cervix	+	++
12	PDNEC, endometrium	++	+
13	PDNEC, gallbladder	++	++
14	PDNEC, lung	++	+++
15	PDNEC, mediastinum	—	0
16	PDNEC, lung	+	++
17	PDNEC, lung	—	0
18	PDNEC, lung	—	0
19	PDNEC, cervix	++	+
20	PDNEC, lung	—	0
21	PDNEC, cervix	—	0
22	PDNEC, lung	—	0
23	PDNEC, cervix	—	0
24	PDNEC, lung	+	++
25	Pheochromacytoma	+++	+++
26	Pheochromacytoma	+++	++++
27	Paraganglioma	+++	++++
28	Paraganglioma	+++	+++
29	Medullary carcinoma, thyroid	+++	++++
29	Medullary carcinoma, thyroid	+++	+++
30	Neuroblastoma	+++	+++
31	Melanocytic nevus, skin	+++	++++
32	Melanocytic nevus, skin	++	+++
33	Melanocytic nevus, skin	+++	++++
34	Melanocytic nevus, skin	++	++++
35	Melanocytic nevus, skin	+++	+++
36	Melanoma, skin	—	0
37	Melanoma, metastatic	—	0

MDNEC, moderately differentiated neuroendocrine carcinoma; PDNEC, poorly differentiated neuroendocrine carcinoma.

was performed on 5-μ m sections from one or two representative areas. The diagnosis of neuroendocrine tumors was confirmed by synaptophysin and chromogranin immunostaining. Immunostain for S-100 and HMB-45 was performed on serial sections to confirm the recognition of olfactory cells, nerve plexuses, and melanocytes of skin, respectively.

Classification of Neuroendocrine Tumors

The neuroendocrine tumors were classified as carcinoid tumor, well-differentiated neuroendocrine carcinoma, moderately differentiated neuroendocrine carcinoma (MDNEC), and poorly differentiated neuroendocrine carcinoma (PDNEC), according to Axiotis (24). A well-differentiated neuroendocrine carcinoma is equivalent to what the World Health Organization (1999) (25) classifies as an atypical carcinoid or to what Gould et al. (26) classifies as a well-differentiated neuroendocrine carcinoma grade I and II, and it is characterized by carcinoidlike growth pattern with an apparent loss of architectural organization (2 to 10 mitoses per high-power field and/or small foci of necrosis). Moderately differentiated neuroendocrine carcinoma is equivalent to atypical carcinoid (World Health Organization) or well-differentiated neuroendocrine carcinoma grade III (Gould et al.), and is it characterized by a carcinoidlike growth pattern with cellular discohesion and a prominent loss of architectural organization, ≥11 mitoses per 10 high-power fields, and conspicuous areas of central necrosis. PDNEC is equivalent to small cell/large cell neuroendocrine carcinoma (World Health Organization) or small cell/intermediate neuroendocrine carcinoma (Gould et al.), and it is characterized by the loss of carcinoidlike growth pattern, numerous mitosis, and geographic necrosis. PDNEC can be small cell type, large cell type, or mixed small/large cell type.

Reagents

A murine monoclonal antibody (sc-7974; Santa Cruz Biotech, Santa Cruz, CA) that recognizes the last 100 C-terminal amino acids of human PTEN protein was used in all immunohistochemical analyzes. The specificity of this antibody has been shown in previous studies (21,27).

Immunostaining

Immunostaining was performed on a Ventana Nexes IHC staining system by using the Nexus Enhanced DAB detection kit Cat#760–003 (Ventana, Tuscon, AZ) according to the manufacturer's recommendations. Deparaffinized, rehydrated 4-μm sections briefly underwent microwave antigen retrieval by using Antigen Retrieval Citra solution (BioGenex, San Ramone, CA). The primary antibody against PTEN protein (1:50 dilution) was incubated at 37°C for 32 minutes. The melanin pigments from nevus, skin, or melanoma tissue sections were removed by standard treatment with 10% hydrogen peroxide for 24–48 hours before histochemical immunostaining (28).

Antigen retrieval

Antigen retrieval techniques have dramatically improved the staining quality of archival tissue sections (29). An immunohistochemical study of PTEN protein expression in prostate carcinoma that used antigen retrieval techniques by heating tissue sections at 100°C for 10 minutes in a pH 6.0-citrate buffer in archival paraffin-embedded tissue sections has been successful (22). We optimized antigen retrieval conditions with a variety of formalin-fixed, paraffin-embedded archival tissues by heating tissue sections at 100°C for 5 minutes in a commercial citrate solution. A comparable staining intensity was achieved by using a commercial immunostaining kit and an automatic stainer.

Controls

Parallel sections incubated with PTEN-blocking peptide/PTEN antibody mixture determined the specificity of PTEN protein immunostaining. In detail, the antibody against PTEN protein was preincubated with excess PTEN-blocking peptide (Santa Cruz Biotech) and parallel slides were stained. Sections were also incubated with the immunoglobulin fraction of normal mouse serum (in place of the primary antibody) in the same protein concentration as the primary antibody.

Quantitation

Cases with staining in more than 10% of cells were considered to have a positive result. PTEN immunostaining was semiquantitatively expressed as percentage of tumor cells stained <10% (0), <25% (1+), <50% (2+), <75% (3+), >75% (4+); and intensity of immunostaining as weak staining (1+), moderate staining (2+), strong staining (3+).

RESULTS

Specificity of PTEN Protein Immunostaining

Sections incubated with the anti-PTEN protein antibody all showed diffuse, granular cytoplasmic immunostaining with positive reactivity, except for a case of PDNEC that showed weak staining in both cytoplasm and nuclei. The parallel sections incubated with the anti-PTEN/PTEN-blocking peptide mixture or the normal mouse serum did not show a reaction product (Fig. 1).

PTEN Immunoreactivity in Fetal and Adult Tissues

The strongest overall PTEN protein immunoreactivity was observed in the central and peripheral nervous systems and in NC/NE tissues. The embryonal, fetal, and adult pituitary glands; pancreatic islets; the adrenal medulla; skin melanocytes; the myenteric and mucosal ganglia of gut; and the paravertebral ganglia stained strongly for PTEN protein (Figs. 2 and 3C). The nerve branches and twigs in peripheral organs and tissues were also strongly positive for PTEN.

PTEN immunostaining in central nervous system (CNS) tissue showed differential expression during human development. In early embryos, the CNS, including the retinal epithelium and primitive nasal olfactory cells, was diffusely and strongly positive for PTEN. Overall, the staining intensity decreases when the fetal CNS matures. Adult brain and spinal cord tissues showed weakly positive staining for PTEN in both astrocytes and neurons (Fig. 3).

In general, PTEN immunostaining results were negative or weak in most nonneural tissues. Blood vessels were the only nonneural/neuroendocrine tissue that showed differential staining for PTEN product. In early fetal development, the aorta and small-vessel wall muscles tested positive for PTEN protein, whereas the adult aorta, medium-size muscular arteries, and small vessels and capillaries lost PTEN protein expression. Adult thyroid glands, breast tissue, basal layers of skin, the esophagus, and the prostate show focal and weak PTEN protein staining.

PTEN Protein Immunoreactivity in NC/NE Tumors

The 37 NC/NE tumors listed in Table 1 were evaluated for PTEN protein immunoreactivity. Figures 4 and 5 are representations of the results obtained. All carcinoid tumors, melanocytic nevi, paragangliomas, pheochromocytomas, medullary thyroid carcinomas, and neuroblastomas showed moderate or strong immunostaining for PTEN protein, and a majority of the tumor cells had positive results (>75%). However, 7 of 13 (54%) PDNECs were negative for PTEN immunostaining; the remaining PDNECs partially lost PTEN expression. Moderately differentiated neuroendocrine carcinomas showed a weak or a moderate PTEN protein stain. The two melanomas examined were also negative for PTEN product.

DISCUSSION

The present immunohistochemical data show 1) differential PTEN expression during CNS development; 2) marked PTEN expression in both fetal and adult NC/NE tissues; and 3) differential PTEN expression in well-differentiated NE tumors and poorly differentiated NE tumors. Because PTEN is not a structural protein, we believe that its expression is important for the differentiation of CNS and NC/NE tissues. PTEN inactivation in a transgenic mouse model results in early embryonic lethality. PTEN+/- and Chimeric mice showed susceptibility to subsequent tumor development, suggesting a dual role in both ontogenic development and tumor suppression (7). However, the formation and development of CNS and NC/NE tissues were not specifically examined in this PTEN knockout animal model. It would be reasonable to speculate that the development of the nervous and vascular systems is mostly likely affected because of the high PTEN expression in these two systems. In addition, Cowden's disease, L'Hermitte-Duclos disease, and Bannayan-Zonana syndrome are characterized by developmental defects such as vascular malformations and features such as dysplastic gangliocytoma of the cerebellum and mental retardation, further suggesting that PTEN plays a role in both neural and vascular development (10–12).

Molecular characterization of PTEN product has shown a high degree of homology to lipid/protein tyrosine phosphatases and tensin (1,30). The lipid phosphatase activity of PTEN product is related to the inhibition of cell cycle progression and G1 arrest through regulation of the PI3K/Akt pathway (31). Akt also is one of the

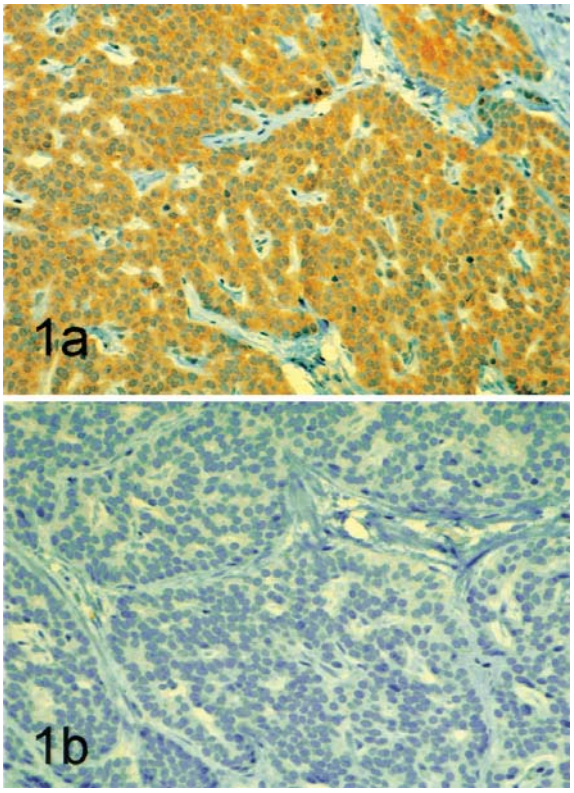

FIG. 1. A: Carcinoid incubated with anti-PTEN monoclonal antibody shows strong PTEN reactivity. **B:** The parallel section incubated with anti-PTEN antibody/blocking peptide mixture shows specific blocking of PTEN immunoreactivity.

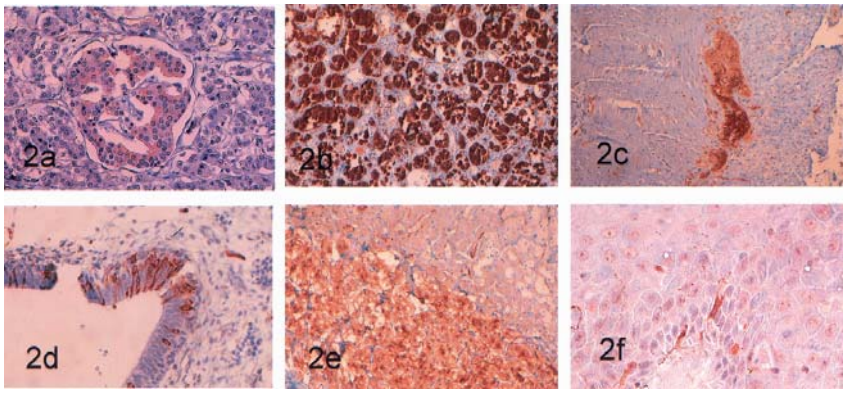

FIG. 2. A: Pancreatic islet. **B:** Adenohypophysis. **C:** Nerve plexus of the intestine. **D:** Olfactory cells of nasal mucosa. **E:** Adrenal medulla. **F:** Skin melanocytes. All show PTEN immunoreactivity.

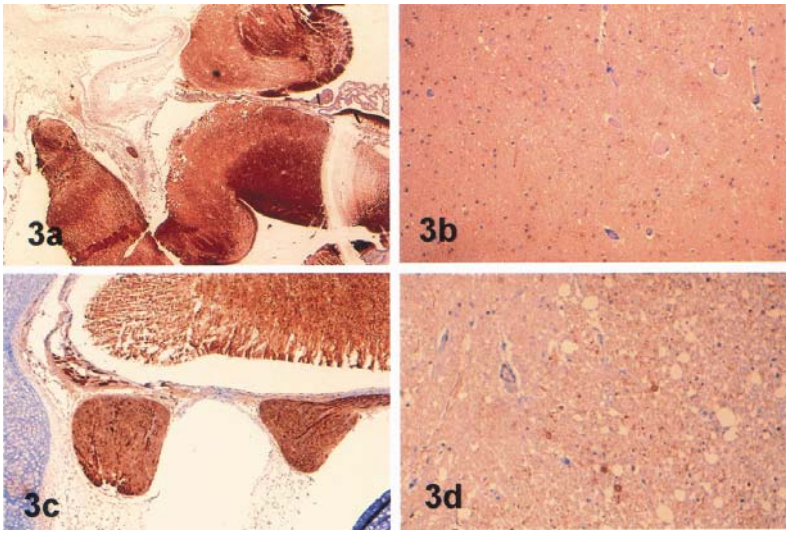

FIG. 3. A and C: Embryonic brain and spinal cord cells (11-week gestational age) show strong PTEN immunoreactivity. **B and D:** Adult brain and spinal cord cells show weak PTEN staining.

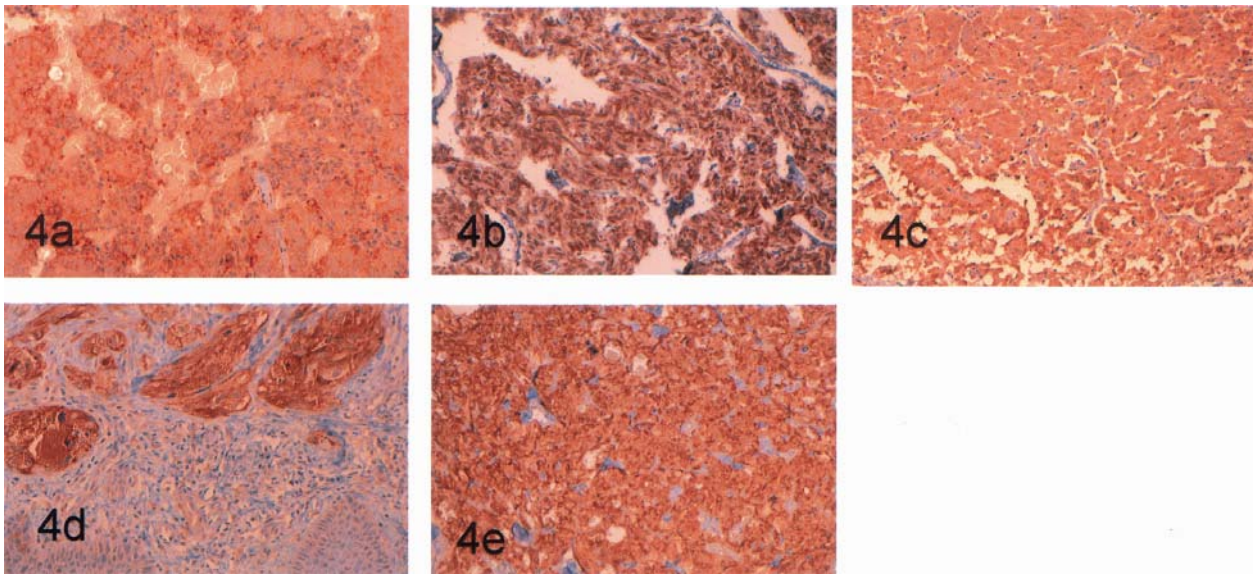

FIG. 4. Positive PTEN immunostaining. **A:** Neuroblastoma. **B:** Pheochromocytoma. **C:** Paraganglioma. **D:** Melanocytic nevus. **E:** Thyroid medullary carcinoma.

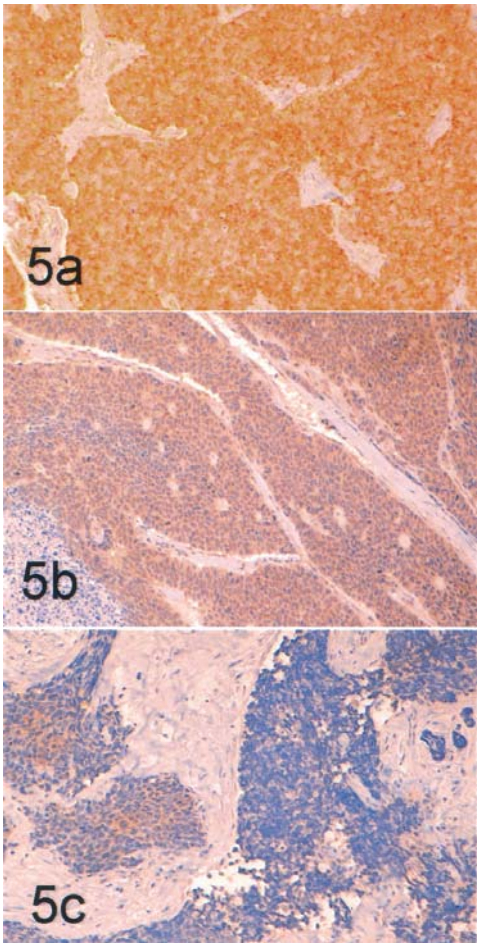

FIG. 5. **A:** Carcinoid shows strong PTEN immunoreactivity. **B:** Moderately differentiated neuroendocrine carcinoma shows moderate PTEN immunoreactivity. **C:** Poorly differentiated neuroendocrine carcinoma shows weak focal PTEN immunoreactivity.

key regulatory molecules involved in the protection of cells against apoptosis (32–34). Tensin binds actin filaments at focal cellular adhesions, integrin-containing complexes, focal adhesion kinase, and growth factor receptors, all of which have been implicated in cell growth regulation, cell mobility, cell-stromal interaction, and angiogenesis (30,35). Because these events are key elements in both tissue developmental differentiation and tumorigenesis, it is not surprising that the highly homologous PTEN protein molecule may play a dual role in both human development and tumor suppression. Actually, a similar characteristic has been found recently in another important molecule for the development of neural crest derivatives, which is encoded by c-ret proto-oncogene (36). It belongs to the membrane receptors of the protein tyrosine kinase family and is expressed during mouse development in a number of tissues, including neural crest derivatives such as dorsal root ganglia, sympathetic and enteric ganglia, and neuroretinal and migrating neural crest cells at earlier stages (37). High levels of c-ret transcripts have been found in pheochromocytomas and medullary thyroid carcinomas, which may be associated with a syndrome called multiple endocrine neoplasia (38). The involvement of c-ret in these tumors has been supported by the identification of mutations in the ret gene. These mutations probably generate dominant tyrosine kinase–activated receptors. Mutations of c-ret have also been found in patients with Hirschsprung's disease, which results from a congenital deficiency in the innervation of the posterior gut (39). The nature of the mutations is different in cancer syndromes and in Hirschsprung disease, in which deletions in the ret gene suggest a loss of function.

PTEN is highly expressed in peripheral nerve ganglia, adrenal medulla, pancreatic islets, pituitary glands, and melanocytes. PTEN staining was not detected in the gastrointestinal and respiratory diffuse neuroendocrine system. The ontogeny of the diffuse neuroendocrine system is still controversial (24). Some studies favor its derivation from endodermal stem cells, whereas others support the theory of migration from the neural crest. The NC stem cells have broad potential in the formation of different tissues, from cartilage to melanocytes. The stepwise restrictions of pluripotent progenitors may result from the expressional regulation of different genes (40, 41). Therefore, it is not surprising that the NC-derived cells do not express PTEN equally, just as chromogranin and synaptophysin are expressed in most NE organs but not in melanocytes (42). In addition, we found no PTEN immunostaining in either the parathyroid gland or its adenoma. The classification of the parathyroid gland as a NE organ has been questioned, although positive immunostaining for synaptophysin has been reported (43).

The regulation of PTEN expression was not well understood until recently when a p53-binding element upstream of the PTEN gene was identified (44). Deletion and mutation analyzes showed that this element is necessary for inducible transactivation of PTEN by p53. As a tumor suppressor, p53 also plays a role in the regulation of cell proliferation and apoptosis. It would be interesting to know how these two tumor suppressors interact in the regulation of cell proliferation and death. A p53-independent element controlling constitutive expression of PTEN was also identified, indicating the complexity of PTEN expression regulation (44).

PTEN is highly expressed in benign and low-grade NE tumors. However, most PDNECs completely lack or partially lose PTEN expression. Loss of PTEN expression is also commonly seen in high-grade prostate and breast carcinomas. PTEN expression is even elevated in benign and low-grade tumors of the prostate and the breast, such as prostatic intraepithelial neoplasia and breast fibroadenoma (20,21,27,45,46). Similarly, the genetic alteration of PTEN is frequent in high-grade glioma but not in low-grade glioma (14,47). These data suggest that loss of the tumor suppressor PTEN product expression is a late event in the progression of these types of tumor. Because the PTEN product functions as a tumor suppressor, loss of or reduction of expression in NE tumors may be a meaningful prognostic marker. Noticeably, the mutation of another tumor suppressor gene, p53, is also considered a late event in most types of carcinoma (48,49). Tumor suppressor genes, such as p53 and the retinoblastoma gene product, have been found in NE neoplasms. The p53 gene is progressively overexpressed in well-differentiated neuroendocrine carcinoma to PDNEC, whereas the retinoblastoma gene is inactivated only in PDNEC (50,51). It is also noted that PTEN is highly expressed in nevus, whereas its expression in two cases of melanoma is lost.

The consistent high expression of PTEN protein in fetal and adult NC/NE tissues and in differentiated NC/NE tumors indicates that PTEN is a NC/NE differentiation marker. PTEN product expression was partially or completely lost in PDNEC. It is noted that some PDNEC only show weak, focal, or even negative immunostain for synaptophysin and/or chromogranin, the two most commonly used NE differentiation markers. We saw two cases of PDNEC that were negative for synaptophysin stain and showed stronger staining for PTEN than for chromogranin. Therefore, we propose PTEN protein immunostain can be used as an adjunct immunohistochemical marker in the diagnosis of NC/NE tumors.

PDNECs are among the most aggressive human neoplasms. Patients often have a dismal prognosis, and a better treatment regimen is certainly desired. PTEN lipid phosphatase activity acts to break cell cycle progression by inhibiting the PI3K pathway (52–54). Activated forms of proto-oncogene Akt were capable of overriding a PTEN-mediated cell cycle block, raising the possibility that Akt might be an important downstream target of PTEN with respect to cell cycle progression (33,55). It has also been shown that cell lines and tumors in which PTEN is lost have elevated levels of activated Akt (56–59). Thus, the loss of immunohistochemical detection of PTEN might predict the presence of activated Akt and, in turn, might become useful as a factor predicting success of therapies directed against this pathway. We also propose that PTEN may be used as a pharmacodiagnostic and prognostic marker.

Although we did not assess the genetic status of PTEN in NE neoplasms, the loss of PTEN protein expression, as assessed by immunohistochemistry, may reflect the majority of the possible mechanisms that result in PTEN inactivation. These would include direct inactivation by homozygous deletion, nonsense mutation, certain internal deletions, and promotor methylation, or indirect inactivation such as loss of a PTEN-directed transcription factor or posttranscriptional modification. Some missense mutations, which do not grossly destabilize the protein product, would not be accounted for by immunohistochemistry. Therefore, we believe immunohistochemistry is the optimal method for evaluating the functional status of PTEN because it would detect a loss of PTEN function induced by a majority of the mechanisms through which gene products are inactivated. □

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 [see comments]. *Science* 1997;275:1943–7.
2. Steck PA, Pershouse MA, Jasser SA, et al. Identification of a candidate tumor suppressor gene, MMAC1, at chromosome

10q23.3 that is mutated in multiple advanced cancers. *Nat Genet* 1997;15:356–62.
3. Huang H, Potter CJ, Tao W, et al. PTEN affects cell size, cell proliferation and apoptosis during Drosophila eye development. *Development* 1999;126:5365–72.
4. Lu Y, Lin YZ, LaPushin R, et al. The PTEN/MMAC1/TEP tumor suppressor gene decreases cell growth and induces apoptosis and anoikis in breast cancer cells. *Oncogene* 1999;18:7034–45.
5. Paramio JM, Navarro M, Segrelles C, et al. PTEN tumor suppressor is linked to the cell cycle control through the retinoblastoma protein. *Oncogene* 1999;18:7462–8.
6. Tian XX, Pang JC, To SS, et al. Restoration of wild-type PTEN expression leads to apoptosis, induces differentiation, and reduces telomerase activity in human glioma cells. *J Neuropathol Exp Neurol* 1999;58:472–9.
7. Di Cristofano A, Pesce B, Cordon-Cardo C, et al. PTEN is essential for embryonic development and tumor suppression. *Nat Genet* 1998;19:348–55.
8. Liaw D, Marsh DJ, Li J, et al. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet* 1997;16:64–7.
9. Marsh DJ, Dahia PL, Zheng Z, et al. Germline mutations in PTEN are present in Bannayan-Zonana syndrome [letter]. *Nat Genet* 1997;16:333–4.
10. Bannayan GA. Lipomatosis, angiomatosis, and macrencephalia. A previously undescribed congenital syndrome. *Arch Pathol* 1971; 92:1–5.
11. Zonana J, Rimoin DL, Davis DC. Macrocephaly with multiple lipomas and hemangiomas. *J Pediatr* 1976;89:600–3.
12. Eng C. Cowden syndrome and LHérmitte-Duclos disease in a family: a single genetic syndrome with pleiotropy. *J Med Genet* 1994; 31:458–61.
13. Matias-Guiu X, Catasus L, Bussaglia E, et al. Molecular pathology of endometrial hyperplasia and carcinoma. *Hum Pathol* 2001;32: 569–77.
14. Davies MP, Gibbs FE, Halliwell N, et al. Mutation in the PTEN/MMAC1 gene in archival low grade and high grade gliomas. *Br J Cancer* 1999;79:1542–8.
15. Duerr EM, Rollbrocker B, Hayashi Y, et al. PTEN mutations in gliomas and glioneuronal tumors. *Oncogene* 1998;16:2259–64.
16. Bussaglia E, del Rio E, Matias-Guiu X, et al. PTEN mutations in endometrial carcinomas: a molecular and clinicopathologic analysis of 38 cases. *Hum Pathol* 2000;31:312–7.
17. Levine RL, Cargile CB, Blazes MS, et al. PTEN mutations and microsatellite instability in complex atypical hyperplasia, a precursor lesion to uterine endometrioid carcinoma. *Cancer Res* 1998; 58:3254–8.
18. Chen J, Lindblom P, Lindblom A. A study of the PTEN/MMAC1 gene in 136 breast cancer families. *Hum Genet* 1998;102:124–5.
19. Chen ST, Yu SY, Tsai M, et al. Mutation analysis of the putative tumor suppression gene PTEN/MMAC1 in sporadic breast cancer. *Breast Cancer Res Treat* 1999;55:85–9.
20. 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 U S A* 1998;95: 5246–50.
21. Perren A, Weng LP, Boag AH, et al. Immunohistochemical evidence of loss of PTEN expression in primary ductal adenocarcinomas of the breast. *Am J Pathol* 1999;155:1253–60.
22. McMenamin ME, Soung P, Perera S, et al. Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. *Cancer Res* 1999;59: 4291–6.
23. Gimm O, Attie-Bitach T, Lees JA, et al. Expression of the PTEN tumor suppressor protein during human development. *Hum Mol Genet* 2000;9:1633–9.
24. Axiotis CA. The neuroendocrine lung. In: Livolsi V, Asa A, eds. *Endocrine Pathology*. Philadelphia: Harcourt, 2002:261–296.
25. World Health Organization. Histological typing of lung and pleural tumors. In: Sobin L, ed. *International Histologic Classification of Tumors*. Geneva, Switzerland: World Health Organization; 1999:1–156.
26. Warren W, Memoli V, Gould V. Well differentiated and small cell neuroendocrine carcinomas of the lung. Two related but distinct clinicopathologic entities. *Virchows Arch B Cell Pathol* 1988;55: 299–310.
27. Depowski P, Rosenthal S, Ross J. Loss of expression of the PTEN gene protein product is associated with poor outcome in breast cancer. *Mod Pathol* 2001;14:672–6.
28. Putt FA. *Manual of Histopathologic Staining Methods*. New York, John Wiley & Sons, 1972.
29. Shi S, Gu J, Jurrens J, Cote R, Taylor C. Development of the antigen retrieval technique: Philosophical and theoretical bases. In: Shi S, Gu J, Taylor C, eds. Antigen retrieval techniques: Eaton Publishing, 2000:17–39.
30. Chen H, Ishii A, Wong WK, Chen LB, Lo SH. Molecular characterization of human tensin. *Biochem J* 2000;351 Pt 2:403–11.
31. 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.
32. Davies MA, Koul D, Dhesi H, et al. Regulation of Akt/PKB activity, cellular growth, and apoptosis in prostate carcinoma cells by MMAC/PTEN. *Cancer Res* 1999;59:2551–6.
33. 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;58:5667–72.
34. Yuan ZQ, Sun M, Feldman RI, et al. Frequent activation of AKT2 and induction of apoptosis by inhibition of phosphoinositide-3-OH kinase/Akt pathway in human ovarian cancer. *Oncogene* 2000;19: 2324–30.
35. Soussi T. PTEN (MMAC, TEP-1): phosphatase and tensin homolog deleted on chromosome 10. *Bull Cancer* 1999;86:715–6.
36. Douarin NM, Dupin E, Ziller C. Genetic and epigenetic control in neural crest development. *Curr Opin Genet Dev* 1994;4:685–95.
37. Pachnis V, Mankoo B, Costantini F. Expression of the c-net proto-oncogene during mouse embryogenesis. *Development* 1993;119: 1005–17.
38. Mulligan LM, Kwok JBJ, Healey CS, et al. Germ-line mutations of the RET proto-oncogene in multiple endocine neoplasia type 2A. *Nature* 1993;363:458–60.
39. Edery P, Lyonnet S, Mulligan LM, et al. Mutations of the RET Proto-oncogene in Hirschsprung's disease. *Nature* 1994;367: 378–80.
40. Bronner-Fraser M. Neural crest cell formation and migration in the developing embryo. *FASEB J* 1994;8:699–707.
41. Vaglia J, Hall BK. Regulation of neural crest cell populations: occurrence, distribution and underlying mechanisms. *Int J Dev Biol* 1999;43:95–110.
42. Gould VE, Wiedemann B, Lee I, et al. Synaptophysin expression in neuroendocrine neoplasms as determined by immunocytochemistry. *Am J Pathol* 1986;126:243–57.
43. Gould VE, Memoli V, Chejfec G, Johannessen JV. The APUD cell system and its neoplasms/Observation on the significance and limitations of the concept. *Surg Clin North Am* 1979;59:93–108.
44. Stambolic V, MacPherson D, Sas D, et al. Regulation of PTEN transcription by p53. *Mol Cell* 2001;8:317–25.
45. Bose S, Wang SI, Terry MB, et al. Allelic loss of chromosome 10q23 is associated with tumor progression in breast carcinomas. *Oncogene* 1998;17:123–7.
46. Garcia JM, Silva JM, Dominguez G, et al. Allelic loss of the PTEN region (10q23) in breast carcinomas of poor pathophenotype. *Breast Cancer Res Treat* 1999;57:237–43.
47. Rasheed BK, Stenzel TT, McLendon RE, et al. PTEN gene mutations are seen in high-grade but not in low-grade gliomas. *Cancer Res* 1997;57:4187–90.
48. Wang L, Yu Y, Sell S. P53 and PCNA as differentiation markers of hepatocellular carcinomas. *J Tumor Marker Oncol* 1998;13: 5–13.
49. Hillstein MC, Sidransky D, Harris CC. P53 mutations in human cancers. *Science* 1991;253:49–53.
50. Barbareschi M, Girlando S, Mauri FA, et al. Tumor suppressor gene products, proliferation, and differentiation markers in lung neuroendocrine neoplasms. *J Pathol* 1992;166:343–50.
51. Harbor JW, Lai SL, Whang-Peng J, et al. Abnormalities in structure and expression of the human retinoblastoma gene in SCLC. *Science* 1997;241:353–7.

52. Hlobilkova A, Guldberg P, Thullberg M, et al. Cell cycle arrest by the PTEN tumor suppressor is target cell specific and may require protein phosphatase activity. *Exp Cell Res* 2000;256:571–7.
53. Maehama T, Dixon JE. PTEN: a tumor suppressor that functions as a phospholipid phosphatase. *Trends Cell Biol* 1999;9:125–8.
54. 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 U S A* 1998;95:13513–8.
55. Sun H, Lesche R, Li DM, et al. PTEN modulates cell cycle progression and cell survival by regulating phosphatidylinositol 3,4,5,-trisphosphate and Akt/protein kinase B signaling pathway. *Proc Natl Acad Sci U S A* 1999;96:6199–204.
56. Dahia PL, Aguiar RC, Alberta J, et al. PTEN is inversely correlated with the cell survival factor Akt/PKB and is inactivated via multiple mechanisms in hematological malignancies. *Hum Mol Genet* 1999;8:185–93.
57. Hyun T, Yam A, Pece S, et al. Loss of PTEN expression leading to high akt activation in human multiple myelomas. *Blood* 2000; 96:3560–8.
58. Wu X, Senechal K, Neshat MS, et al. The PTEN/MMAC1 tumor suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase/Akt pathway. *Proc Natl Acad Sci U S A* 1998;95:15587–91.
59. Zhang P, Steinberg BM. Overexpression of PTEN/MMAC1 and decreased activation of Akt in human papillomavirus-infected laryngeal papillomas. *Cancer Res* 2000;60:1457–62.