 Human Molecular Genetics, 2000, Vol. 9, No. 2 283–287

Involvement of *PTEN* mutations in the genetic pathways of colorectal cancerogenesis

Ginevra Guanti[+,§], Nicoletta Resta[+], Cristiano Simone, Filomena Cariola[1], Ignazio Demma[2], Paola Fiorente[1] and Mattia Gentile[1]

Dipartimento di Medicina Interna e Medicina Pubblica, Sezione di Genetica Medica, Policlinico, Piazza Giulio Cesare, 70124 Bari, Italy, [1]Genetica Medica and [2]Divisione di Chirurgia, IRCCS 'De Bellis', 70013 Castellana, Italy

Received 17 September 1999; Revised and Accepted 17 November 1999

So far, somatic mutations of the *PTEN* gene have been found in several different neoplasms but not in colorectal tumours. As exons 7 and 8 of the *PTEN* coding sequence contain an $(A)_6$ repeat and mononucleotide repeat sequences are targets for mutations in tumours with microsatellite instability (MI), we screened a panel of sporadic colorectal tumours exhibiting MI to test whether *PTEN* gene repeats are frequently mutated in MI$^+$ colorectal cancers. Of 32 cases studied, seven mutations were found in six (18.75%) patients, as a *PTEN* biallelic frameshift mutation was observed in one case, with consequent loss of function of the gene. Loss of heterozygosity, evaluated in the remaining five cases using the microsatellite marker *D10S541*, was detected in two of three informative samples. To further address the role of the *PTEN* gene in MI$^+$ colorectal cancer, in the six patients with mutated *PTEN*, we analysed the mononucleotide repeats of six other genes: *BAX*, *hMSH3*, *hMSH6*, *TGFbRII*, *IGFIIR* and *APC*. In two of these six patients, mutations of the *TGFbRII* gene only were present, indicating that *PTEN* may have a role in the mutator pathway of colorectal tumorigenesis. Overall, these results indicate that *PTEN* mutations are selected for during tumorigenesis in MI$^+$ colorectal tumours. The mutation of both *PTEN* alleles and evidence that the *PTEN* protein is expressed in normal colon suggest that loss of function of this gene could play a direct role in tumorigenesis.

INTRODUCTION

PTEN is a candidate tumour suppressor gene that has recently been isolated and localized at chromosomal band 10q23 (1–3). This gene has three different names: *PTEN* (phosphatase and tensin homologue deleted on chromosome ten) (1), *MMAC1* (mutated in multiple advanced cancers 1) (2) and *TEP1* (TGF-β-regulated and epithelial cell-enriched phosphatase 1) (3), and codes for the 403 amino acid protein with tyrosine phosphatase activity. It contains a region of homology to tensin and auxillin, cytoskeletal proteins that interact with adhesion molecules. Germ-line mutations of *PTEN* are responsible for Cowden syndrome (4), also known as multiple hamartoma syndrome, and Bannayan–Zonana syndrome (5) whose features include lipoma and haemangiomas. Somatic mutations of the gene have been found in prostate, endometrial, breast and brain tumours but seem to be a relatively rare event in colorectal cancer (6–9).

As exon 7 and 8 *PTEN* sequences contain an $(A)_6$ repeat and mononucleotide repeat sequences are frequent targets for mutations in endometrial tumours with microsatellite instability (MI$^+$) (6,10), we decided to screen a panel of sporadic colorectal tumours exhibiting genetic instability, to test whether these repeats in the *PTEN* gene are mutational targets in MI$^+$ colorectal cancer.

RESULTS

A total of 32 MI$^+$ tumours were analysed. The PCR products of exons 7 and 8 of *PTEN* were sequenced and seven frameshift mutations in the $(A)_6$ tracts were found in six samples, as in one case (T186) the same tumour showed two different mutations, in exon 7 and in exon 8, respectively. RT–PCR analysis of the tumour mRNA of this patient showed that of eight plasmids three contained an $(A)_5$ tract in exon 7 and a wild-type mononucleotide repeat in exon 8 and five contained a wild-type $(A)_6$ tract in exon 7 and a frameshift mutation in the $(A)_6$ tract of exon 8. In addition to the A deletion in the $(A)_6$ tract of exon 8, one of these plasmids contained a missense mutation (N310Q), also in exon 8 (Fig. 1).

The frameshift mutations observed in the six tumour samples were either a 1 bp deletion in the $(A)_6$ repeat of exon 7 or a 1 bp deletion in the $(A)_6$ repeat of exon 8 (Fig. 2) and both created a premature stop, at codon 275 of exon 7 and codon 343 of exon 8, respectively. A summary of the mutational analysis of the two *PTEN* exons is shown in Table 1.

To assess the role of *PTEN* gene mutations in colorectal cancer, we first examined the presence of the *PTEN* mRNA in normal colonic mucosa. To increase the sensitivity of our analysis of RNA expression, we performed RT–PCR assays using the primer pair reported by Wang *et al.* (11) to avoid the amplification of a *PTEN* pseudogene. As predicted, a 671 bp product was obtained

[+]These authors contributed equally to this work
[§]To whom correspondence should be addressed. Tel: +39 0805478270; Fax: +39 0805478269; Email: guanti@medgene.uniba.it

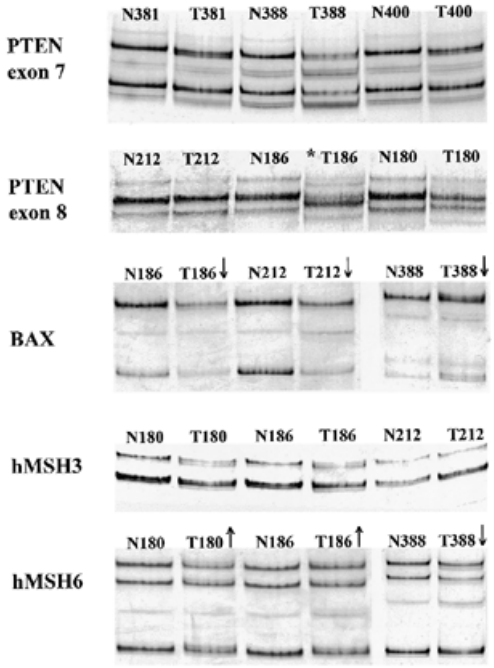

Figure 1. SSCP analysis. Representative examples of frameshift mutations at coding sequence repeats within the *PTEN*, *BAX*, *hMSH3* and *hMSH6* genes. All the tumor samples of *PTEN* exon 7 and two (T180 and T186) of exon 8 contain the deletion of 1 bp. In addition, sample T186 contains a missense (N310Q) mutation. Arrows pointing up or down indicate insertions or deletions of one nucleotide, respectively. T, tumor tissue DNA; N, normal tissue DNA from the same patient.

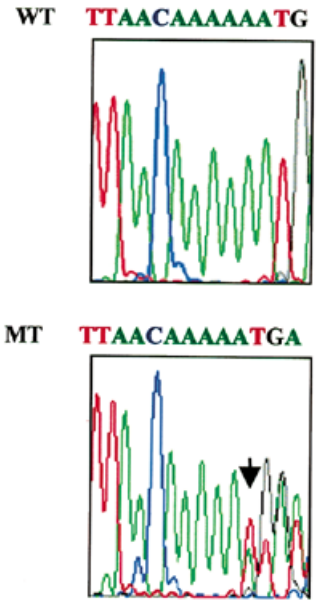

Figure 2. Portion of electropherograms illustrating the *PTEN* exon 8 frameshift mutation (MT) found in patient T180 (bottom) and the wild-type sequence (WT) from the same patient (top). The arrow indicates the deleted nucleotide (A).

from normal colonic mucosa and from normal prostate tissue used as control (data not shown).

Because mRNA expression of tumour suppressor genes does not necessarily reflect protein expression, we aimed to assess the presence of the *PTEN* protein in normal colonic mucosa using a goat polyclonal antibody raised against the N-terminus of human *PTEN* protein. A band migrating at 55 kDa was detected in the western blot of whole cell extracts (Fig. 3) prepared from normal colonic cells, as well as in extracts of normal prostate tissue recently demonstrated to contain the *PTEN* protein (11–12). All together, these results indicate that the *PTEN* gene is expressed in normal colonic mucosa.

We then decided to investigate the role of *PTEN* mutations in the 'mutator pathway' of tumorigenesis in MI^+ colorectal cancer, searching for the presence of instability in genes whose mutations contribute to uncontrolled growth and progression to malignancy. The region comprising mononucleotide repeats of *BAX*, *hMSH3*, *hMSH6*, *TGFbRII*, *IGFIIR* and *APC* genes was characterized in tumours and corresponding normal tissues from these six patients. Table 1 shows the results of the analysis. *BAX* frameshift mutations were detected in patients T186, T212 and T388 (Fig. 1); patients T180 and T186 showed mutations in both the *hMSH3* and *hMSH6* genes (Fig. 1). Mutations of the *TGFbRII* gene were present in all of the six patients analyzed in the region of the poly(A) sequence whereas the GT repeat region was normal in all of them; mutations of *IGFIIR* and *APC* genes were not detected in any of the patients.

Because *MLH1* and *MSH2* gene mutations are the most frequent cause of DNA instability in colorectal tumours, we screened the entire coding sequence of these two genes for mutations in the three carriers of *PTEN* mutations whose tumour DNA was available. In patient T180 one A deletion was found at nucleotide 77 in the first exon of *MLH1*; no mutations were identified in the *MLH1* and in the *MSH2* gene of the other two patients.

The pathology reports for these six patients with *PTEN* mutations did not show any characteristic features. Patient T186, a female, had a stage II moderately differentiated adenocarcinoma of the caecum; patients T212, T381, T388 and T400 had a stage II poorly differentiated adenocarcinoma of the proximal colon; patient T180 had two synchronous stage II moderately differentiated adenocarcinomas of the rectum. The latter patient had been surgically treated for an adenocarcinoma of the caecum 20 years previously.

DISCUSSION

Several lines of evidence suggest that functional inactivation of *PTEN* is required in the pathogenesis of tumours of various histological origins. Although frequently occurring in glioblastoma, prostate and endometrium sporadic cancers, *PTEN* gene mutations seem to be a relatively rare event in colorectal cancer. Since there are two sites containing an $(A)_6$ repeat in the *PTEN* coding sequence and mononucleotide repeat sequences constitute frequent targets for mutations in tumours with microsatellite instability (MI^+), we analysed a panel of colorectal tumours exhibiting MI for frameshift mutations to assess whether mutational inactivation of *PTEN* contributes to colorectal tumorigenesis.

Seven mutations of the *PTEN* gene, all of which consisted of the deletion of 1 bp in the $(A)_6$ tract of exon 7 or of exon 8 resulting in a stop codon and subsequent premature termination, were found in 6 of 32 (18.75%) cases studied. Cancers with mutated *PTEN* did not show a striking clinical phenotype.

Table 1. Status of coding mononucleotide repeats within the *PTEN, BAX, hMSH3, hMSH6, IGFIIR, TGFbRII* and *APC* genes in the tumour DNA of the six patients

Case	*PTEN* Exon 7 $(A)_6$	*PTEN* Exon 8 $(A)_6$	BAX $(G)_8$	hMSH3 $(A)_8$	hMSH6 $(C)_8$	IGFIIR $(G)_8$	TGFβRII $(A)_{10}$	APC $(A)_7$	APC[a] $(A)_6$	APC[b] $(A)_6$
T180	wt	5/6	wt	7/8	8/9	wt	9/10	wt	wt	wt
T186	5/6	6/5	7/8	7/8	8/9	wt	9/10	wt	wt	wt
T212	5/6	wt	7/8	wt	wt	wt	8/9	wt	wt	wt
t381	5/6	wt	wt	wt	wt	wt	9/10	wt	wt	wt
t388	5/6	wt	8/9	wt	7/8	wt	9/10	wt	wt	wt
t400	5/6	wt	wt	wt	wt	wt	9/10	wt	wt	wt

wt, wild-type.
[a]$(A)_6$ at nucleotide 5370.
[b]$(A)_6$ at nucleotide 5385.

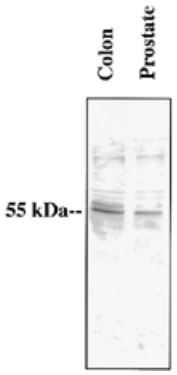

Figure 3. *PTEN* protein expression. Western blot analysis performed using a goat anti-human *PTEN* polyclonal antibody to probe the whole cell extracts from normal colonic mucosa and prostate tissue. A band with an apparent molecular mass of 55 kDa is identified by the antibody in both the tissues.

In one of the six patients, T186, a *PTEN* biallelic frameshift mutation was observed, i.e. a deletion in the $(A)_6$ tract of exon 7 in one allele and a deletion in the $(A)_6$ tract of exon 8 in the other allele, with consequent loss of function of the gene. We did not have enough DNA or fresh tissue available from the other five patients to perform a mutation search in the coding sequence of the *PTEN* gene to detect the mutation of the second allele, according to Knudson's two hits theory. We therefore screened DNA isolated from paraffin blocks for loss of heterozygosity (LOH), using the linked microsatellite marker *D10S541*. Three samples were informative and two of these, T180 and T388, showed LOH.

To gain a better understanding of the role of *PTEN* gene function in colonic cells, we checked the expression of the *PTEN* gene at both RNA and protein level. Our data demonstrate that the *PTEN* protein is present in normal colonic cells.

Then, to address the role of *PTEN* mutations in the mutator pathway of colorectal tumorigenesis, the six patients with alterations of the *PTEN* gene were analysed for the presence of frameshift mutations in the mononucleotide repeats of six other genes: *TGFbRII*, *hMSH3*, *hMSH6*, *BAX*, *IGFIIR* and *APC*, whose mutations contribute to progression towards malignancy in colorectal cancer. Interestingly, in patients T381 and T400 mutations of the *TGFbRII* gene only were present, indicating that *PTEN* probably plays a role in the mutator pathway of colorectal tumorigenesis.

In Cowden and Bannayan syndrome patients (13–14), exons 7 and 8, believed to encode potential tyrosine kinase phosphorylation sites of the *PTEN* protein, are the second hot spot for mutations. However, it should be noted that the great majority of germ-line mutations responsible for these two hereditary syndromes occur in different sites on exons 7 and 8 from those of the poly(A) repeats, reflecting different mutational mechanisms. Cowden syndrome is characterized by intestinal hamartomatous polyps but no studies to date have succeeded in demonstrating their progression to colorectal carcinoma. Interestingly, colon cancer has been described in *Pten*$^{+/-}$ chimeric mice (15).

Mutations of the *PTEN* gene in colorectal cancers had not been observed in previous reports (6–9). However, all of these studies analysed unselected patients. Because MI has been observed in 12–15% of sporadic colorectal cancers and mutations of the *PTEN* gene in 18% of MI$^+$ colorectal tumours, when analysing unselected colorectal tumours *PTEN* alterations would be expected in ~2.7% of cases. Therefore, a negative result is likely unless the sample studied is big enough.

Overall, our results indicate that *PTEN* mutations are selected for during tumorigenesis in MI$^+$ colorectal tumours; in addition, the evidence that the *PTEN* protein is expressed in colonic cells, as well as the presence of mutations in both alleles of the *PTEN* gene, provide support to the idea that loss of function of the gene plays a direct role in tumorigenesis.

Although the mechanisms through which *PTEN* mutations contribute to cancer are not yet entirely clear, several studies emphasize the importance of *PTEN* in normal cell homeostasis. *PTEN* possesses lipid phosphatase activity and a tyrosine phosphatase domain, the latter being responsible for a decrease in fibronectin-mediated focal adhesion kinase phosphorylation (16). Thus, *PTEN* may act as a regulator of cell surface interactions with the extracellular matrix through its protein phosphatase activity and as a controller of cell growth through its lipid phosphatase activity, which could, in turn, downregulate protein kinase B (PKB)/serine/threonine kinase encoded by the akt proto-oncogene (AKT) (17). It has recently (18) been shown that *PTEN*-deficient cells exhibit a reduced ability to trigger apoptosis when they receive a death signal, accompanied by constitutively increased levels of PKB/AKT phosphorylation. Changes in the phosphorylation level of this

latter protein are crucial in regulating cell survival by conferring resistance to *PTEN*-induced apoptosis. On the basis of these data it seems reasonable to speculate that *PTEN* mutations are not simply bystander events but play an active role in promoting abnormal growth of colorectal cells.

The data presented here aim to contribute to elucidating the function of the *PTEN* gene and its role in the so-called mutator pathway of tumorigenesis.

MATERIALS AND METHODS

Tumour samples

A total of 32 MI^+ but otherwise unselected colorectal tumours, paired with adjacent normal tissues, were analysed. All the tumour tissue samples were obtained at the surgery and freshly frozen. Gross cryostat dissection was limited to areas consisting mainly of neoplastic cells. Germ-line DNA was available either from peripheral blood or normal adjacent colon tissue from each patient.

High molecular weight genomic DNA was extracted from frozen tissues or blood according to the standard methods. MI was assessed using five polymorphic markers: *BAT25*, *BAT26*, *D2S123*, *D5S346* and *D18S858*. MI was considered present when two or more of the five markers showed instability.

Mutational analysis of *PTEN*

Mutations of *PTEN* at repetitive sequences within its coding region were identified using a PCR-based assay. The 229 bp region encompassing the *PTEN* poly$(A)_6$ repeat beginning at nucleotide 795 of exon 7 and the 200 bp region encompassing the poly$(A)_6$ repeat beginning at position 963 of exon 8 were amplified using the primers reported by Risinger *et al.* (10). PCR was carried out for 30 cycles, each cycle consisting of denaturation for 20 s at 95°C, annealing for 20 s at 48°C, and extension for 30 s at 72°C. Mutations were searched by single-strand conformation polymorphism (SSCP) analysis; after electrophoresis the gel bands were visualized by the silver staining method; bands with mobility alterations were gel- and column-purified and directly sequenced using the ABI Ready Reaction Dye Terminator Cycle Sequencing kit according to the manufacturer's instructions (Perkin Elmer, Norwalk, CT) and the 377 ABI Prism Sequencer. All the tumours with variant SSCP bands were sequenced in forward and reverse orientations. All mutations were confirmed by duplicate sequencing of a newly amplified product. Determination of the presence of the mutation in the same or different alleles was addressed by PCR amplification of the *PTEN* mRNA using the following primers: 5′-ATCCTCAGTTTGTGGTCT-3′ and 5′-GGAGAAAAGTATCGGTTGGC-3′. The PCR products, corresponding to the 382 bp region encompassing the poly$(A)_6$ repeats of exon 7 and exon 8, were cloned into plasmid vectors (TOPO-TA cloning kit; Invitrogen, Groningen, The Netherlands) and several clones sequenced as before.

LOH analysis

Total genomic DNA purified from paraffin-embedded sections was screened for LOH using the polymorphic marker *D10S541* flanking the *PTEN* locus.

Analysis of *BAX*, *hMSH3*, *hMSH6*, *TGFbRII*, *IGFIIR* and *APC* mononucleotide repeats

The presence of frameshift mutations in the following genes: *BAX*, *hMSH3*, *hMSH6*, *TGFbRII*, *IGFIIR* and *APC*, containing mononucleotide repeats was studied. The 94 bp region encompassing the *BAX*$(G)_8$ tract at nucleotides 114–121, the 92 bp region of *IGFIIR* containing poly$(G)_8$ at nucleotides 4089–4096, the poly$(C)_8$ and poly$(A)_8$ repeats at codons 1116–1118, respectively, of the *hMSH6* gene and at codons 381–383 of *hMSH3*, and the $(A)_{10}$ microsatellite sequence at nucleotides 709–718 of *TGFbRII* were characterized using the primers and the PCR conditions as described by Yamamoto *et al*. (19). The PCR products were characterized by electrophoresis on denaturing 6% polyacrylamide sequencing gels and by SSCP, followed by sequencing of bands with mobility alterations.

In addition, the $(A)_7$ tract at the nucleotide 6573 and the two $(A)_6$ repeats at nucleotides 5370 and 5385, respectively, of the *APC* gene were analysed. The amplification was performed using the primers for the fragments L and P of exon 15 (20). The PCR products were then sequenced.

Mutational analysis of *MSH1* and *MLH2* genes

The promoter regions and each exon of *MLH1* and *MSH2* genes from genomic DNA were individually amplified and subsequently sequenced directly.

Western blot analysis

Specimens of normal colonic mucosa and prostate tissue were diced into very small pieces and homogenized with a dounce homogenizer. The cells were lysed in a solution containing 0.5% Nonidet P40, 50 mM Tris–HCl pH 8, 110 mM NaCl, 0.5% Triton X-100 and proteinase inhibitors, 2 mM PMSF, 0.5 μg/ml leupeptin. Lysates consisting of 150 μg of protein were separated by SDS–PAGE (10%) and transferred to Immobilon P membranes (Millipore, Bedford, MA). The membranes were then probed with anti*PTEN*(N-19) polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:700 dilution according to the manufacturer's protocol. After overnight incubation, detection was performed using alkaline phosphatase-conjugated secondary antibody visualized by incubation in NBT/BCIP developing solutions.

ACKNOWLEDGEMENTS

We thank Dr Alessandro Stella for helpful discussion and Mary V.C. Pragnell for careful linguistic text revision. This work was partially supported by MURST (ex 40%) and AIRC. The experiments performed here complied with the current laws in Italy.

REFERENCES

1. Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S.I., Puc, J., Miliaresis, C., Rodgers, L., McCombie, R. *et al*. (1997) *PTEN*, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science*, **275**, 1943–1947.
2. Steck, P.A., Pershouse, M.A., Jasser, S.A., Yung, W.K., Lin, H., Ligon, A.H., Langford, L.A., Baumgard, M.L., Hattier, T., Davis, T. *et al*. (1997) Identification of a candidate tumour suppressor gene, *MMAC1*, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nature Genet.*, **15**, 356–362.

3. Li, D.M. and Sun, H. (1997) *TEP1*, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor β. *Cancer Res.*, **57**, 2124–2129.
4. Liaw, D., Marsh, D.J., Li, J., Dahia, P.L., Wang, S.I., Zheng, Z., Bose, S., Call, K.M., Tsou, H.C., Peacocke, M. *et al*. (1997) Germline mutations of the *PTEN* gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nature Genet.*, **16**, 64–67.
5. Marsh, D.J., Dahia, P.L., Zheng, Z., Liaw, D., Parsons, R., Gorlin, R.J. and Eng, C. (1997) Germline mutations in *PTEN* are present in Bannayan–Zonana syndrome. *Nature Genet.*, **16**, 333–334.
6. Kong, D., Suzuki, A., Zou, T., Sakurada, A., Kemp, L., Wakatsuki, S., Yokoyama, T., Yamakawa, S., Furukawa, T., Sato, M. *et al*. (1997) *PTEN1* is frequently mutated in primary endometrial carcinomas. *Nature Genet.*, **17**, 143–144.
7. Wang, Z.J., Taylor, F., Churchman, M., Norbury, G. and Tomlinson, I. (1998) Genetic pathways of colorectal carcinogenesis rarely involve the *PTEN* and *LKB1* genes outside the inherited hamartoma syndromes. *Am. J. Pathol.*, **153**, 363–366.
8. Okami, K., Wu, L., Riggins, G., Cairns, P., Goggins, M., Evron, E., Halachmi, N., Ahrendt, S.A., Reed, A.L., Hilgers, W. *et al*. (1998) Analysis of *PTEN*/*MMAC1* alterations in aerodigestive tract tumors. *Cancer Res.*, **58**, 509–511.
9. Chang, J.G., Chen, Y.J., Perng, L.I., Wang, N.M., Kao, M.C., Yang, T.J., Chang, C.P. and Tsai, C.H. (1999) Mutation analysis of the *PTEN/MMAC1* gene in cancers of the digestive tract. *Eur. J. Cancer*, **35**, 647–651.
10. Risinger, J.I., Hayes, A.K., Berchuck, A. and Barrett, J.C. (1997) *PTEN*/*MMAC1* mutations in endometrial cancers. *Cancer Res.*, **57**, 4736–4738.
11. Wang, Y.E., Wu, X., Suzuki, H., Reiter, R.E., Tran, C., Vessella, R., Said, J.W., Isaac, B.W. and Sawyers, C. (1998) Inactivation of the tumor suppressor *PTEN/MMAC1* in advanced human prostate cancer through loss of expression. *Proc. Natl Acad. Sci. USA*, **95**, 5246–5250.
12. McManemin, M.E., Soung, P., Perera, S., Kaplan, I., Loda, M. and Sellers, W.L. (1999) Loss of *PTEN* expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. *Cancer Res.*, **59**, 4291–4296.
13. Marsh, D.J., Coulon, V., Lunetta, K.L., Rocca-Serra, P., Dahia, P.M.L., Zheng, Z., Liaw, D., Caron, S., Duboué, B., Lin, A.Y. *et al*. (1998) Mutation spectrum and genotype–phenotype analyses in Cowden disease and Bannayan–Zonana syndrome, two hamartoma syndromes with germline *PTEN* mutation. *Hum. Mol. Genet.*, **7**, 507–519.
14. Marsh, D.J., Kum, J.B., Lunetta, K.L., Bennett, M.J., Gorlin, R.J., Ahmed, S.F., Bodurtha, J., Crowe, C., Curtis, M.J., Dasouki, M. *et al*. (1999) *PTEN* mutations spectrum and genotype–phenotype correlations in Bannayan–Riley–Ruvalcaba syndrome suggest a single entity with Cowden syndrome. *Hum. Mol. Genet.*, **8**, 1461–1472.
15. Di Cristofano, A., Pesce, B., Cordon-Cardo, C. and Pandolfi, P.P. (1998) *PTEN* is essential for embryonic development and tumor suppression. *Nature Genet.*, **19**, 348–355.
16. Tamura, M., Gu, J., Matsumoto, K., Aota, S., Parsons, R. and Yamada, K.M. (1998) Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor *PTEN*. *Science*, **280**, 1614–1617.
17. Maehama, T. and Dixon, J.E. (1998) The tumor suppressor, *PTEN*/*MMAC1*, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-triphosphate. *J. Biol. Chem.*, **273**, 13375–13378.
18. Stambolic, V., Suzuki, A., de la Pompa, J.L., Brothers, G.M., Mirtsos, C., Sasaki, T., Ruland, J., Penninger, J.M., Siderovski, D.P. and Mak, T.W. (1998) Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor *PTEN*. *Cell*, **95**, 29–39.
19. Yamamoto, H., Sawai, H. and Perucho, M. (1997) Frameshift somatic mutations in gastrointestinal cancer of microsatellite mutator phenotype. *Cancer Res.*, **57**, 4420–4426.
20. Groden, J., Thliveris, A., Samowitz, W., Carlson, M., Gelbert, L., Albertsen, H., Joslyn, G., Stevens, J., Spirio, L., Robertson, M. *et al*. (1991) Identification and characterization of the familial adenomatous polyposis coli gene. *Cell*, **66**, 589–600.