Mutations of *PTEN/MMAC1* in Primary Prostate Cancers from Chinese Patients[1]

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ABSTRACT

***PTEN/MMAC1* is a putative tumor suppressor gene located on 10q23, one of the most frequently deleted chromosomal regions in human prostate cancer. Although mutations of *PTEN* have often been detected in metastases of prostate cancer, localized tumors have shown lower rates of mutation, which have varied from 0 to 20% among different studies. It is unknown whether the rate of *PTEN* mutations is different in prostate cancer from Asian men compared with Western men. To further clarify the role of *PTEN* in prostate cancer and to examine the gene for mutations in Asian men, we analyzed 32 cases of primary prostate cancers from Chinese patients, each of whom was not diagnosed by screening with serum prostate-specific antigen, for *PTEN* mutations using the methods of tissue microdissection, single-strand conformational polymorphism, and direct DNA sequencing. Seventy % of the tumors were Gleason scores 8–10, whereas the remainder were Gleason score 7. Six metastases of prostate cancer from American patients were also analyzed. Five of 32 (16%) primary prostate cancers from Chinese men and two of six metastases from American men showed mutations in a total of 10 codons of *PTEN*, which involved exons 1, 2, 5, 8, and 9. Two of the mutations were truncation type, whereas the rest were missense mutations. The mutation frequency in these cases from Asian patients was higher than that in our previous study of cases in radical prostatectomy specimens from American men, in which the 40 primary tumors were lower grade and had been detected by serum prostate-specific antigen test. We conclude that mutation of *PTEN* occurs more often in primary prostate cancers of Chinese men, whose tumors are high grade and reflective of an unscreened population.**

INTRODUCTION

A candidate tumor suppressor gene designated *PTEN*, *MMAC1*, or *TEP-1* (referred to as *PTEN* hereafter) was identified (1–3) from the q23.3 region of chromosome 10, one of the most frequently deleted regions in prostate cancer (4). The *PTEN* gene has nine exons that encode a 403-amino acid protein of a dual-specific phosphatase with putative actin-binding and tyrosine phosphatase domains. Introduction of PTEN into cancer cells that lack *PTEN* function inhibits cell migration and induces cell cycle arrest and apoptosis via negative regulation of the phosphatidylinositol 3′-kinase/protein kinase B/Akt signaling pathway (5–7). Mutation and down-regulation of the *PTEN* gene have been detected in various human cancers including that of the prostate (8–10). In addition, germ-line mutations in *PTEN* are associated with Cowden disease (11), in which patients are at increased risk for certain cancers.

Thus far, *PTEN* appears to be the most frequently mutated gene in metastases of prostate cancer, occurring in at least 1 metastatic site in 12 of 19 (63%) patients who had multiple metastases (12) and in 9 of 15 (60%) cell lines and xenografts primarily derived from metastases of prostate cancer (13). These results indicate a role for *PTEN* in the progression of prostate cancer. Mutations of *PTEN* in localized prostate cancers have been found at lower frequencies including 1 of 28 (4%; Ref. 14), 1 of 25 (4%; Ref. 15), 1 of 40 (2.5%; Ref. 16), 0 of 45 (17), and 1 of 22 (5%; Ref. 18). Somewhat higher rates of mutations have been observed in other studies including 10 of 80 [12.5%; 10 of 23 (43%) in cases with loss of heterozygosity at *PTEN*; Ref. 19], 5 of 37 (13.5%; Ref. 20), 8 of 60 (13%; Ref. 21), and 1 of 10 (10%; Ref. 9). In hereditary prostate cancer, the role of *PTEN* has not been detected (22, 23).

The incidence of prostate cancer is lower in Asian men compared with Western men, but the specific genetic or environmental factors that are important are unknown (24, 25). Obviously, more cancers are detected in Western men because of screening with serum PSA[3] test. The frequency of PTEN mutations in prostate cancer from Asian men has been little studied. One study of 45 primary prostate cancers from Japanese patients did not detect any *PTEN* mutation (17). In this study, we analyzed primary prostate cancers from 32 Chinese patients, who were not diagnosed using the PSA test. Rather, they were diagnosed after showing clinical symptoms. We also analyzed six metastases from American patients who died of prostate cancer to document additional PTEN mutations in fatal prostatic disease.

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[3] The abbreviations used are: PSA, prostate-specific antigen; SSCP, single-strand conformational polymorphism.

MATERIALS AND METHODS

Tumor Samples. Thirty-two formalin-fixed, paraffin-embedded prostate cancer specimens from radical prostatectomy from previously untreated Chinese patients were used in this study. These patients went to physicians after showing various symptoms of prostate cancer, *e.g.*, difficulty in voiding, urodynia, urgent and frequent urination, and hematuria. None of them were involved in PSA screening. Their prostates were examined by one or more of the following means: rectal ultrasound detection, digital rectal examination, computed tomography, and magnetic resonance imaging. Biopsy was performed for the patients who were suspected to have prostate cancer, and only those whose cancers were at stages B–C underwent radical prostatectomy. The prostatectomies were performed by four surgeons over a period of 5 years. All specimens were from archived paraffin blocks that had been used in routine diagnosis of cancer, and none of them were collected specifically for this study. In addition, DNA was available from six distant metastases from American patients who died of prostate cancer. The clinicopathological characteristics of the tumors are listed in Table 1. The exact tumor stage for the Chinese patients was not available. Tumor cells for DNA isolation were collected from 7 μm H&E-stained sections by microdissection using a protocol described previously (26), which typically ensured a minimum of 70% neoplastic cells for each sample. Nonneoplastic cells collected were present on the same slides as cancer cells and included stromal cells, lymphocytes, and urothelium; in most cases, they did not include nonneoplastic prostatic epithelium. For the cases of metastases, nonneoplastic cells were collected from lymph nodes or seminal vesicles. Use of the human specimens in this study was approved by the institutional human investigation committee.

PCR-SSCP Analysis. Each of the primary prostate cancers was first screened for mutation by using the PCR-SSCP approach. Primers used for each *PTEN* exon were the same as described previously (16). PCRs for the SSCP contained 5–10 ng of genomic DNA, 1× PCR buffer [20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100, and 0.1 mg/ml BSA], 1 μM of each primer, 3 μM of each deoxynucleotide triphosphate, 1 μCi of [α-^{32}P]dCTP (3000 Ci/mmol), 0.6 unit of Taq DNA polymerase, and 0.1 unit of Pfu DNA polymerase and was incubated at 95°C for 5 min followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. The ^{32}P-labeled PCR products were electrophoresed at 5 W overnight at room temperature in a 6% nondenaturing polyacrylamide gel in 1× TPE buffer [30 mM Tris, 20 mM PIPES (1,4-piperazinediethanesulfonic acid), and 1 mM Na_2EDTA (pH 6.8)] as described previously (27). PCR products were also analyzed in a 0.25× MDE gel (FMC BioProducts, Rockland, ME) containing 10% glycerol, which was also run at 5 W overnight at room temperature. After electrophoresis, the gels were dried and exposed to X-ray film for 1–2 days. Samples showing a bandshift for a specific exon were reamplified for both tumor DNA and matched nonneoplastic cells to confirm the shift, using the same conditions.

DNA Sequencing. For the samples which repeatedly showed a bandshift in the SSCP analysis, shifted bands were cut and immersed in 20 μl of H_2O, following the protocol described by Kukita *et al.* (27). Two μl of the released DNA were amplified by PCR using the same primers, as in SSCP analysis, in a 50-μl of reaction. The PCR conditions were the same except that 200 μM of each deoxynucleotide triphosphate and no [^{32}P]dCTP were used. These PCR products were purified by using the QIAquick PCR purification kit (Qiagen, Valencia, CA), and were sequenced by using the ThermoSequenase Cycle Sequencing kit (USB) following the manufacturer's instructions. Sequencing data were collected and analyzed by using the ScanDNASIS and MacDNASIS software (Hitachi Software, San Bruno, CA).

For the six metastases of prostate cancer, which tended to be more homogeneous in neoplastic cells, their DNAs were amplified by PCR for each of the *PTEN* exons, and the resultant PCR products were purified and directly sequenced by the same procedure as described above. For an exon showing a mutation,

Table 1 Clinicopathological characteristics of prostate cancer specimens analyzed and mutation status of *PTEN* in each case

Case no.	Patient Age (yr)	Gleason score	*PTEN* mutation
80	NA[a]	7	No
82	52	9	No
83	64	8	No
84	51	10	No
85	NA	10	No
86	61	8	No
89	61	8	Exon 5 (T418C, polymorphism)
90	63	8	No
91	NA	9	Exon 2 (A1197G, R55G)
92	NA	8	Exon 5 (T302A, I101A; A403G, I135V)
95	60	10	No
96	65	8	No
98	NA	7	No
99	83	7	No
100	75	9	No
101	NA	9	No
102	NA	7	No
103	NA	9	No
104	75	9	No
105	74	9	No
107	83	NA	No
108	71	NA	No
109	NA	10	Exon 5 (A449G, Q150G)
110	65	9	No
111	70	7	No
113	67	7	Exon 1 (G58T, G20Stop)
114	59	8	Exon 8 (C814T, H272Y)
116	66	7	No
117	NA	8	Exon 9 (A1086G, polymorphism)
119	56	9	No
120	NA	7	No
121	66	7	No
42[b]	77	Lymph node	No
46[b]	75	Liver	No
47[b]	70	Lymph node	Exon 9 (A1031G, K344R; C1043T, T348I; A1144T, T382S)
48[b]	73	Lymph node	No
49[b]	75	Lymph node	Exon 5 (C328T, Q110Stop)
51[b]	66	Liver	No

[a] NA, not available.
[b] Lymph node and liver were the organ sites of metastases.

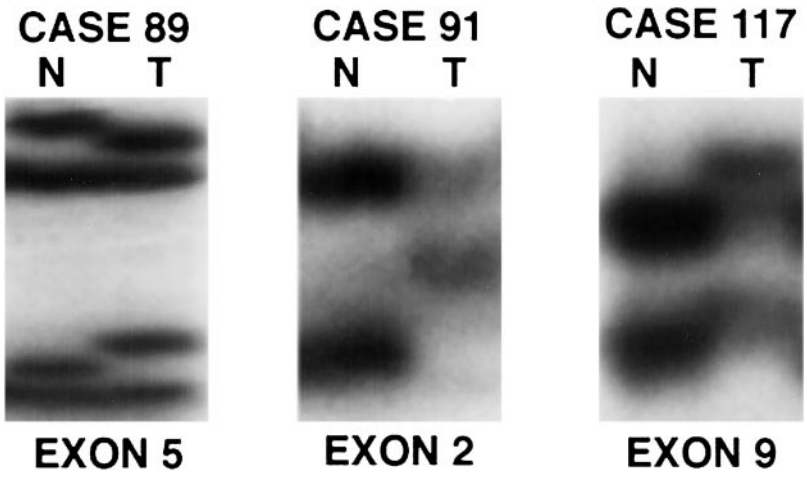

Fig. 1 Examples of SSCP analysis of *PTEN* in prostate cancer specimens. *N* and *T*, nonneoplastic and tumor cells, respectively. For each example, the case number is indicated at the *top*, and exon number is at the *bottom*. Each case has shifted bands in their tumor cells compared with nonneoplastic cells.

the PCR sequencing procedure was repeated to confirm the mutations. Once confirmed, matched normal DNA for a specific exon was also amplified by PCR and sequenced to determine whether a mutation was somatic or germ line.

Statistical Analysis. The difference in the frequency of *PTEN* mutations between primary tumors in the current study and that of our previous study (16) was analyzed statistically by the use of Fisher's exact test (two-tailed; Ref. 28).

RESULTS

Seventy % of the 32 primary prostate cancers from Chinese men were Gleason scores 8–10, whereas 30% were Gleason score 7. PCR-SSCP and direct DNA sequencing analyses of these samples revealed *PTEN* sequence alterations in 7 cases. Examples of bandshifts for tumors in SSCP assay, which indicated the existence of sequence alterations in the *PTEN* gene, are shown in Fig. 1, and examples of DNA sequencing ladders that identify *PTEN* mutations are shown in Fig. 2. Tumor cases and their *PTEN* mutation status are listed in Table 1. Although 2 of the 7 cases had alterations that did not change the PTEN polypeptide, five cases (16%) had mutations that could potentially change PTEN function (Table 1). Case 113 had a nonsense mutation at codon 20 that would truncate the majority of the PTEN protein. Case 92 had two missense mutations in its exon 5, which changed codons 101 and 135 from isoleucine to alanine and valine, respectively. Cases 91, 109, and 114 showed missense mutations that changed codons 55, 150, and 272 from arginine, glutamine, and histidine to glycine, glycine, and tyrosine, respectively.

We also analyzed six metastases of prostate cancer from American men, using the methods of PCR amplification and direct DNA sequencing. Two cases showed *PTEN* mutations. Case 49 had a nonsense mutation at codon 110 in exon 5 that would truncate the PTEN protein, and case 47 had three missense mutations in exon 9 of *PTEN*, changing codon 344 from lysine to arginine, codon 348 from threonine to isoleucine, and codon 382 from threonine to serine.

Each of the above mutations was somatic, as the matched nonneoplastic cells showed no mutations. The difference in the frequency of 16% for *PTEN* mutation in the cancers from Chinese patients compared with the frequency of 2.5% in our prior analysis of 40 resected primary tumors detected in American men after PSA test and biopsy (16) showed a trend in significance ($P = 0.08$).

DISCUSSION

The *PTEN* gene was isolated from the q23 region of chromosome 10, one of the most frequently deleted regions in prostate cancer (4, 29, 30). Mutations of the gene have been detected in various human cancers including that of the prostate (9, 12, 13, 19–21), implicating *PTEN* in the development and/or progression of prostate cancer. It is thus far the most frequently mutated gene in prostate cancer. Our finding of *PTEN* mutations in 5 of 32 primary, high-grade prostate cancer specimens confirms that *PTEN* is a major gene, if not the target gene, for the 10q23 region of deletion in a subset of prostate cancers.

Mutation frequencies of *PTEN* in prostate cancer differ among studies, largely because of differences in tumor grade and stage in the study populations. Mutations up to 60% have been detected in studies of prostate cancer cell lines and xenografts from metastases (13), whereas in some studies of localized disease, few or no mutations have been detected (16, 17). In this study, we detected *PTEN* mutations in 5 of 32 (16%) primary prostate cancers from Chinese patients who were diagnosed with clinical symptoms but without the aid of the serum PSA screening test. This frequency was higher than that (1 of 40 or 2.5%) detected in primary prostate cancers from American patients who were diagnosed by PSA test in our previous study (16). The majority of tumors from the Chinese patients were high grade (Gleason scores 8–10), whereas the majority of tumors in the American patients were lower grade (Gleason scores 5–7), indicating that PTEN mutations occur more often in tumors with high Gleason scores, even in those that are primary lesions. This conclusion is consistent with published studies of primary prostate cancers (15, 17, 20). In one study of 37 primary tumors with 20 (54%) high-grade and 17 (46%) lower grade lesions, five cases, four of which were high-grade tumors, had PTEN mutations (20). In another study of 45 primary tumors that were mainly low-grade cancers [30 (67%) lower grade cases and 15 (33%) high-grade cases], no PTEN mutations were

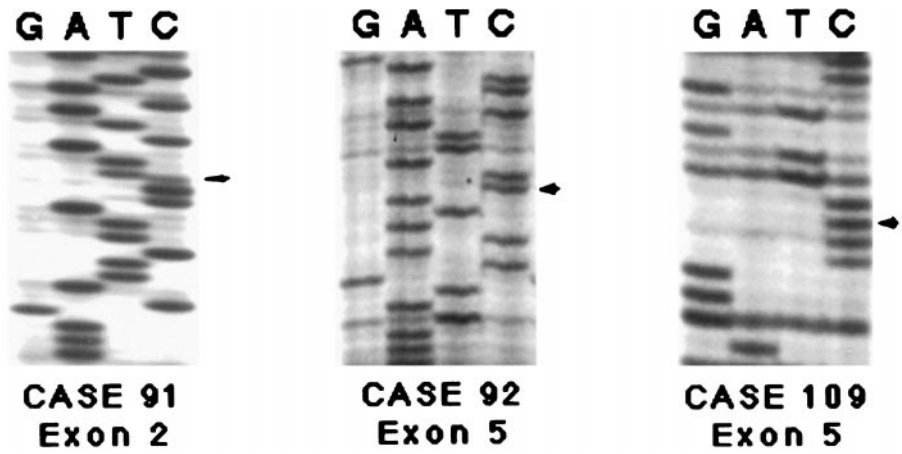

Fig. 2 Examples of sequencing analysis of shifted bands from SSCP experiments. *Arrow*, mutated nucleotide; *bottom*, case number and exon number.

found (17). Summarizing five studies in which both tumor grade and PTEN mutations were available (15–17, 20), we found that 9 of 67 (13.4%) high-grade tumors showed PTEN mutations, whereas only 3 of 117 (2.6%) lower grade cases showed mutations. The former rate is significantly higher than the latter ($P = 0.01$) using the χ^2 analysis-of-contingency table (28). Consistent with mutation studies, loss of PTEN expression has also been shown to correlate with high grade of primary prostate cancer (9, 10).

It has been reported that prostate cancer incidence is lower in Asian men than in Western men (24, 25). Although one study of Japanese patients did not detect any *PTEN* mutations in 45 primary tumors that were mainly low-grade cancers (17), we found more frequent *PTEN* mutations in a group of Chinese patients that had mainly high-grade tumors in this study; the latter is consistent with studies in Western men (20). These results suggest that *PTEN* is likely not a genetic factor contributing to the racial difference in prostate cancer incidence. This conclusion is further supported by the fact that all of the *PTEN* mutations were detected in prostate cancer cells only and not in their matched nonneoplastic cells. Also, no *PTEN* mutation has been detected in familial prostate cancers (22, 23). The differences in PTEN mutation rates in our study compared with that of Orikasa *et al.* (17) may be attributable to differences in the distribution of tumor grades between the study samples.

We detected multiple mutations for *PTEN* in two tumors, *i.e.*, case 92 had two missense mutations in exon 5 and case 47 had three missense mutations in exon 9 (Table 1). The heterogeneous nature of prostate cancer is well known (31); therefore, it is likely that multiple mutations of *PTEN* in one tumor may come from different subclones of tumor cells. In an analysis of metastases involving multiple organ sites in patients who died of prostate cancer, Suzuki *et al.* (12) found that different metastases within the same patient had different PTEN mutation status, indicating a complex genetic relationship between various subclonal lineages of prostate cancer cells. Mutation of exon 5 appears to be more frequent than that of other exons in both Cowden disease and various somatic cancers (8).

In summary, *PTEN* mutations were seen more often in primary prostate cancers from Chinese men compared with localized tumors from American patients. This difference is likely attributable to the presence of an excess of high-grade cancers in the Chinese patients. Whether primary prostate tumors with *PTEN* mutations have a greater proclivity to metastasize than those of similar grade and stage without mutations remains to be determined.

REFERENCES

1. Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S. I., Puc, J., Miliaresis, C., Rodgers, L., McCombie, R., Bigner, S. H., Giovanella, B. C., Ittmann, M., Tycko, B., Hibshoosh, H., Wigler, M. H., and Parsons, R. *PTEN*, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science (Washington DC), *275:* 1943–1947, 1997.

2. Steck, P. A., Pershouse, M. A., Jasser, S. A., Yung, W. K. A., Lin, H., Ligon, A. H., Langford, L. A., Baumgard, M. L., Hattier, T., Davis, T., Frye, C., Hu, R., Swedlund, B., Teng, D. H. F., and Tavtigian, S. V. Identification of a candidate tumour suppressor gene, *MMAC1*, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat. Genet., *15:* 356–362, 1997.

3. Li, D. M., and Sun, H. 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, 1997.

4. Gray, I. C., Phillips, S. M., Lee, S. J., Neoptolemos, J. P., Weissenbach, J., and Spurr, N. K. Loss of the chromosomal region 10q23–25 in prostate cancer. Cancer Res., *55:* 4800–4803, 1995.

5. Davies, M. A., Koul, D., Dhesi, H., Berman, R., McDonnell, T. J., McConkey, D., Yung, W. K., and Steck, P. A. Regulation of Akt/PKB activity, cellular growth, and apoptosis in prostate carcinoma cells by MMAC/PTEN. Cancer Res., *59:* 2551–2556, 1999.

6. Persad, S., Attwell, S., Gray, V., Delcommenne, M., Troussard, A., Sanghera, J., and Dedhar, S. Inhibition of integrin-linked kinase (ILK) suppresses activation of protein kinase B/Akt and induces cell cycle arrest and apoptosis of PTEN-mutant prostate cancer cells. Proc. Natl. Acad. Sci. USA, *97:* 3207–3212, 2000.

7. Wu, X., Senechal, K., Neshat, M. S., Whang, Y. E., and Sawyers, C. L. The PTEN/MMAC1 tumor suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase/Akt pathway. Proc. Natl. Acad. Sci. USA, *95:* 15587–15591, 1998.

8. Ali, I. U., Schriml, L. M., and Dean, M. Mutational spectra of *PTEN/MMAC1* gene: a tumor suppressor with lipid phosphatase activity. J. Natl. Cancer Inst., *91:* 1922–1932, 1999.

9. Whang, Y. E., Wu, X., Suzuki, H., Reiter, R. E., Tran, C., Vessella, R. L., Said, J. W., Isaacs, W. B., and Sawyers, C. L. Inactivation of the tumor suppressor PTEN/MMAC1 in advanced human prostate cancer through loss of expression. Proc. Natl. Acad. Sci. USA, *95:* 5246–5250, 1998.

10. McMenamin, M. E., Soung, P., Perera, S., Kaplan, I., Loda, M., and Sellers, W. R. Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. Cancer Res., *59:* 4291–4296, 1999.

11. Liaw, D., Marsh, D. J., Li, J., Dahia, P. L. M., Wang, S. I., Zheng, Z. M., Bose, S., Call, K. M., Tsou, H. C., Peacocke, M., Eng, C., and Parsons, R. Germline mutations of the *PTEN* gene in Cowden-disease, an inherited breast and thyroid cancer syndrome. Nat. Genet., *16:* 64–67, 1997.

12. Suzuki, H., Freije, D., Nusskern, D. R., Okami, K., Cairns, P., Sidransky, D., Isaacs, W. B., and Bova, G. S. Interfocal heterogeneity of *PTEN/MMAC1* gene alterations in multiple metastatic prostate cancer tissues. Cancer Res., *58:* 204–209, 1998.

13. Vlietstra, R. J., van Alewijk, D. C., Hermans, K. G., van Steenbrugge, G. J., and Trapman, J. Frequent inactivation of PTEN in prostate cancer cell lines and xenografts. Cancer Res., *58:* 2720–2723, 1998.

14. Facher, E. A., and Law, J. C. PTEN and prostate cancer. J. Med. Genet., *35:* 790, 1998.

15. Feilotter, H. E., Nagai, M. A., Boag, A. H., Eng, C., and Mulligan, L. M. Analysis of PTEN and the 10q23 region in primary prostate carcinomas. Oncogene, *16:* 1743–1748, 1998.

16. Dong, J. T., Sipe, T. W., Hyytinen, E. R., Li, C. L., Heise, C., McClintock, D. E., Grant, C. D., Chung, L. W., and Frierson, H. F., Jr. PTEN/MMAC1 is infrequently mutated in pT2 and pT3 carcinomas of the prostate. Oncogene, *17:* 1979–1982, 1998.

17. Orikasa, K., Fukushige, S., Hoshi, S., Orikasa, S., Kondo, K., Miyoshi, Y., Kubota, Y., and Horii, A. Infrequent genetic alterations of the *PTEN* gene in Japanese patients with sporadic prostate cancer. J Hum. Genet., *43:* 228–230, 1998.

18. Pesche, S., Latil, A., Muzeau, F., Cussenot, O., Fournier, G., Longy, M., Eng, C., and Lidereau, R. PTEN/MMAC1/TEP1 involvement in primary prostate cancers. Oncogene, *16:* 2879–2883, 1998.

19. Cairns, P., Okami, K., Halachmi, S., Halachmi, N., Esteller, M., Herman, J. G., Isaacs, W. B., Bova, G. S., and Sidransky, D. Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. Cancer Res., *57:* 4997–5000, 1997.

20. Gray, I. C., Stewart, L. M., Phillips, S. M., Hamilton, J. A., Gray, N. E., Watson, G. J., Spurr, N. K., and Snary, D. Mutation and expression analysis of the putative prostate tumour-suppressor gene *PTEN*. Br. J. Cancer, *78:* 1296–1300, 1998.

21. Wang, S. I., Parsons, R., and Ittmann, M. Homozygous deletion of the *PTEN* tumor suppressor gene in a subset of prostate adenocarcinomas. Clin. Cancer Res., *4:* 811–815, 1998.

22. Cooney, K. A., Tsou, H. C., Petty, E. M., Miesfeldt, S., Ping, X. L., Gruener, A. C., and Peacocke, M. Absence of PTEN germ-line mutations in men with a potential inherited predisposition to prostate cancer. Clin. Cancer Res., *5:* 1387–1391, 1999.

23. Forrest, M. S., Edwards, S. M., Hamoudi, R. A., Dearnaley, D. P., Arden-Jones, A., Dowe, A., Murkin, A., Kelly, J., Teare, M. D., Easton, D. F., Knowles, M. A., Bishop, D. T., and Eeles, R. A. No evidence of germline PTEN mutations in familial prostate cancer. J. Med. Genet., *37:* 210–212, 2000.

24. Gu, F. L., Xia, T. L., and Kong, X. T. Preliminary study of the frequency of benign prostatic hyperplasia and prostatic cancer in China. Urology, *44:* 688–691, 1994.

25. Angwafo, F. F. Migration and prostate cancer: an international perspective. J. Natl. Med. Assoc., *90:* S720–S723, 1998.

26. Moskaluk, C. A., and Kern, S. E. Microdissection and polymerase chain reaction amplification of genomic DNA from histological tissue sections. Am. J. Pathol., *150:* 1547–1552, 1997.

27. Kukita, Y., Tahira, T., Sommer, S. S., and Hayashi, K. SSCP analysis of long DNA fragments in low pH gel. Hum. Mutat, *10:* 400–407, 1997.

28. Glantz, S. A. Primer of Biostatistics, p. 473. New York: McGraw-Hill, 1997.

29. Atkin, N. B., and Baker, M. C. Chromosome study of five cancers of the prostate. Hum. Genet., *70:* 359–364, 1985.

30. Arps, S., Rodewald, A., Schmalenberger, B., Carl, P., Bressel, M., and Kastendieck, H. Cytogenetic survey of 32 cancers of the prostate. Cancer Genet. Cytogenet., *66:* 93–99, 1993.

31. Macintosh, C. A., Stower, M., Reid, N., and Maitland, N. J. Precise microdissection of human prostate cancer reveals genotypic heterogeneity. Cancer Res., *58:* 23–28, 1998.

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