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PTEN/MMAC1 Mutation and Frequent Loss of Heterozygosity Identified in Chromosome 10q in a Subset of Hepatocellular Carcinomas

Yoshiyuki Fujiwara,[1,4] Dave S. B. Hoon,[3] Terumasa Yamada,[1] Koji Umeshita,[1] Mitsukazu Gotoh,[1] Masato Sakon,[1] Isamu Nishisho[2] and Morito Monden[1]

[1]Department of Surgery II, Osaka University Medical School, 2-2 Yamada-oka, Suita-city, Osaka 565-0871, [2]Department of Surgery, Osaka National Hospital, 2-1-14 Hoenzaka, Cyuo-ku, Osaka-city, Osaka 540-0006 and [3]Department of Molecular Oncology, John Wayne Cancer Institute, 2200 Santa Monica Blvd., Santa Monica, CA 90404, USA

Frequent allelic losses on chromosome 10q have been reported in several types of cancers, suggesting the presence of a putative tumor suppressor gene(s) on the chromosomal arm. We examined loss of heterozygosity (LOH) on chromosome 10q in 37 hepatocellular carcinomas (HCC) using eleven dinucleotide microsatellite markers, spanning the entire chromosome arm of 10q. Twelve (32%) out of 37 informative cases showed allelic losses of at least one locus on 10q and eight tumors showed a partial deletion of 10q. Analysis of deletion mapping of these eight cases identified two commonly deleted regions within the distal part of 10q (10q24-q26), a 20-cM interval flanked by D10S597 and D10S216 and a 24-cM interval flanked by D10S216 and D10S590. Moreover, we detected a somatic missense mutation (Met→Val) of a candidate tumor suppressor gene *PTEN/MMAC1*, located at 10q23.3, in one HCC with LOH of 10q. Our findings indicated the presence of putative tumor suppressor gene(s) in the distal region of 10q that might be involved in the development and progression of HCC. Inactivation of *PTEN/MMAC1* gene located outside the commonly deleted region of 10q might also play an important role in a subset of HCCs.

Key words: PTEN/MMAC-1 — Hepatocellular carcinoma — LOH — Microsatellite marker

Loss of tumor suppressor gene(s) is involved in the development and progression of certain cancers. Recent studies have shown that alterations in several tumor suppressor genes and putative tumor suppressor loci are involved in development and progression of hepatocellular carcinoma (HCC), including *p53* gene located on 17p13,[1] *RB1* on 13q14,[2] 1p, 4q, 5q, 8p, 11p, 16q and 22q.[3–9] Mutations of protooncogenes such as *Ras* and *β-catenin* have also been described, although the percentage of HCC showing such defects is small.[10,11]

Frequent loss of heterozygosity (LOH) on chromosome 10q has been reported in various types of human cancers such as glioma, lung cancer, bladder cancer, non-Hodgkin lymphoma, malignant meningioma, melanoma, endometrial cancer, thyroid cancer, and prostate.[12–21] These findings indicate that one or more putative tumor suppressor genes are located on this chromosomal arm.

A new putative tumor suppressor gene *PTEN/MMAC1* was recently isolated from the chromosomal region 10q23.3 and somatic mutations of this gene have been reported in a variety of human cancers such as glioma, endometrial cancer, breast cancer, thyroid cancer, lung cancer, bladder cancer and prostate cancer.[22–29] This gene was also identified as being responsible for two hamartoma syndromes; Cowden disease and Bannayan-Riley-Ruvalcaba syndrome.[30–32]

Although frequent LOHs on 10q have been reported in HCC using a limited number of markers, chromosome 10q is among the least characterized in HCC.[7,8] Therefore, we examined in the present study the involvement of *PTEN/MMAC1* mapped to 10q23.3, in the progression of HCC. Analysis of mutations of this new putative tumor suppressor gene in HCC DNAs was performed by single strand conformation polymorphism (SSCP) and direct sequencing analyses.

MATERIALS AND METHODS

Specimens and DNA extraction Thirty-seven pairs of tumors and corresponding non-cancerous tissues were obtained from 37 patients with HCC at the Department of Surgery II, Osaka University Medical School. All specimens had been previously examined histopathologically to confirm the diagnosis. Tumors were histologically classified as well differentiated, moderately differentiated, poorly differentiated, and undifferentiated carcinoma, according to the typing scheme of the Japanese Liver Cancer Society.[33] After surgical removal, specimens were carefully dissected and stored at −80°C until extraction

[4] To whom correspondence should be addressed.
E-mail: fujiwara@surg2.med.osaka-u.ac.jp

of DNA. High-molecular-weight genomic DNAs were extracted from frozen tissues according to the method described previously.[34)]

LOH analysis using microsatellite markers Ten primer sets for polymerase chain reaction (PCR) amplification of microsatellite markers were selected to span the whole chromosome arm according to the Centre d'Etude du Polymorphism Humain (CEPH)/Genethon linkage map.[35)] We also used D10S2491, a newly isolated microsatellite marker from the locus of *PTEN/MMAC1*.[23)] The linear order of CA-repeat microsatellite markers used is (centromere)–D10S561–D10S202–D10S2491–D10S564–D10S192–D10S597–D10S587–D10S216–10S212–D10S590–D10S212–D10S555–(telomere) (Table I). One of each pair of primer sets was labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase (Promega, Madison, WI). Genomic DNA (100 ng) was amplified by PCR in a 10-μl reaction mixture containing 1× PCR buffer (6.7 m*M* Tris, 16.6 m*M* ammonium sulfate, 6.7 m*M* EDTA, 10 m*M* β-mercaptoethanol), 20 pmol each of [γ-^{32}P]ATP-labeled primer and non-labeled primer, 1 U of *Taq* DNA polymerase, 0.8 m*M* dNTP mixtures, and 1.5 m*M* of $MgCl_2$. Thirty-five PCR cycles were performed with each cycle consisting of 30 s at 94°C, 30 s at 50–55°C, and 30 s at 72°C in a Gene Amp PCR system 9600 (Perkin-Elmer Cetus, Norwalk, CT). Five microliter volume of each solution, mixed with an equal volume of loading dye (95% formamide, 10 m*M* EDTA, 0.02% xylene cyanol and 0.02% bromophenol blue), was denatured and then electrophoresed in 6% polyacrylamide gel containing 7.7 *M* urea and 32% formamide. Gels were dried and exposed to X-ray film for 4–48 h.

Determination of LOH Evaluation of LOH was performed by comparing the intensity of two alleles in informative cases using a scanning densitometer. Tumors were scored as exhibiting LOH if there was an absence or >50% reduction in intensity of one allele in the tumor sample compared to the normal allele.

Table I. Frequency of Loss of Heterozygosity (LOH) at Different Microsatellite Loci on Chromosome 10q

Microsatellite marker	Informative cases	Cases with LOH	% of LOH
D10S561	14	1	7
D10S202	19	2	11
D10S2491	26	4	15
D10S564	18	3	17
D10S192	19	5	26
D10S592	21	5	24
D10S587	15	4	27
D10S216	26	5	19
D10S590	15	5	33
D10S212	18	6	33
D10S555	19	5	26

Mutation analysis SSCP analysis was performed by PCR amplification of each of the nine exons, corresponding to the coding region of *PTEN/MMAC1* gene. PCR primers were designed in introns to amplify each whole exon and intron-exon boundary. The nucleotide sequences of PCR primers have been described previously[36)] except primers for exon 5, which were designed as follows: forward primer, 5′-CTTATTCTGAGGTTATCTTTTTACC-3′; reverse primer, 5′-CTCAGAATCCAGGAAGAGGA-3′. PCR reactions were performed in 10-μl solutions containing 50 ng of genomic DNA, 2 pmol of each primer set, and 0.5 U of *Taq* I polymerase. SSCP analysis was performed using 5% nondenaturing polyacrylamide gels (acrylamide:N,N-bisacrylamide 99:1) containing 10% glycerol. After electrophoresis for 16 h at 4°C, the gels were stained with Silver Green II (TaKaRa, Otsu) and scanned with a fluorescence image analyzer (FMBio II Multi-View, TaKaRa). PCR products showing a mobility shift in SSCP analysis were subjected to sequence analysis using a Direct Sequencing Kit ("BioDye," PE Applied Biosystems, Warrington, UK).

RESULTS

LOH analysis of HCC DNAs from 37 HCCs were examined for LOH using a panel of eleven microsatellite markers spanning the whole region of chromosome 10q. Table I lists all dinucleotide repeat markers used for LOH analysis, as well as the frequency of LOH at each locus. The frequency of LOH was higher at the locus of the marker closer to the telomeric region of 10q. The frequency of LOH was highest at D10S212 and D10S590 (6 of 18, 5 of 15), and lowest at D10S561 (1 of 14, 7%). Four out of 26 (15%) tumors showed LOH at the *PTEN/MMAC1* locus (D10S2491). All 37 cases were informative with two or more markers and 12 (32%) showed allelic losses of at least one locus on 10q. Among 12 tumors with LOH, eight showed LOH at some loci, whereas the others showed retention of heterozygosity, indicating partial or interstitial deletions of the long arm of chromosome 10 in these tumors. Representative autoradiograms of cases with partial or interstitial deletions are shown in Fig. 1. Tumor biopsy #117 showed LOH at D10S212, but heterozygosity at D10S216 (Fig. 1a). Tumor biopsy #127 showed LOH at D10S587, but retained heterozygosity at D10S590 (Fig. 1b). Fig. 2 summarizes the results of LOH analysis with 11 markers in 12 cases with LOH. All samples showed LOH at the distal part of 10q. Two commonly deleted regions in the distal part were located in a 20-cM interval flanked by D10S597 and D10S216, and a 24-cM interval between D10S590 and D10S216, as shown by bars with arrows in Fig. 2.

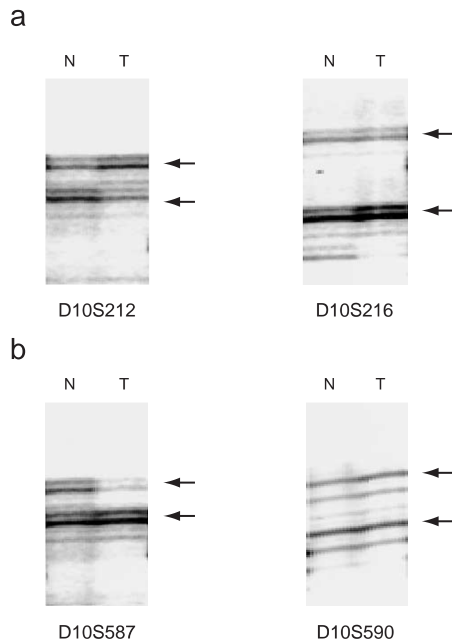

Fig. 1. Autoradiograms of LOH analyses of hepatocellular tumors showing partial or interstitial deletions on chromosome 10q. a, tumor biopsy #117; b, tumor biopsy #127. Microsatellite markers are listed underneath each autoradiogram of paired DNA from HCC tumors (T) and corresponding normal tissues (N). Arrowheads indicate polymorphic bands.

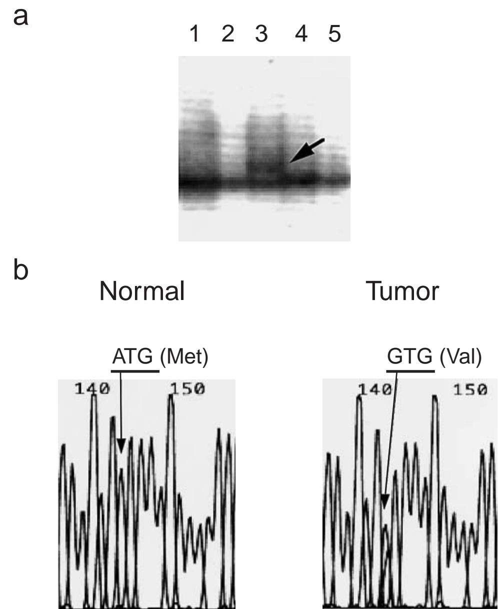

Fig. 3. a: SSCP analysis of the *PTEN* gene in HCC tumors. The arrowhead indicates the abnormally shifted band in lane 3 (tumor biopsy #134). The presence of the shifted band was confirmed by a repeated experiment. b: Sequence analysis of the *PTEN/MMAC1* gene PCR product showing the shifted band in SSCP analysis. The normal base pair of the sequence (A) was altered to (G) in tumor biopsy #134, resulting in substitution of GTG (Val) for ATG (Met). The sequence (A) remaining in tumor DNA may be produced by amplification of genomic DNA from normal cell contamination.

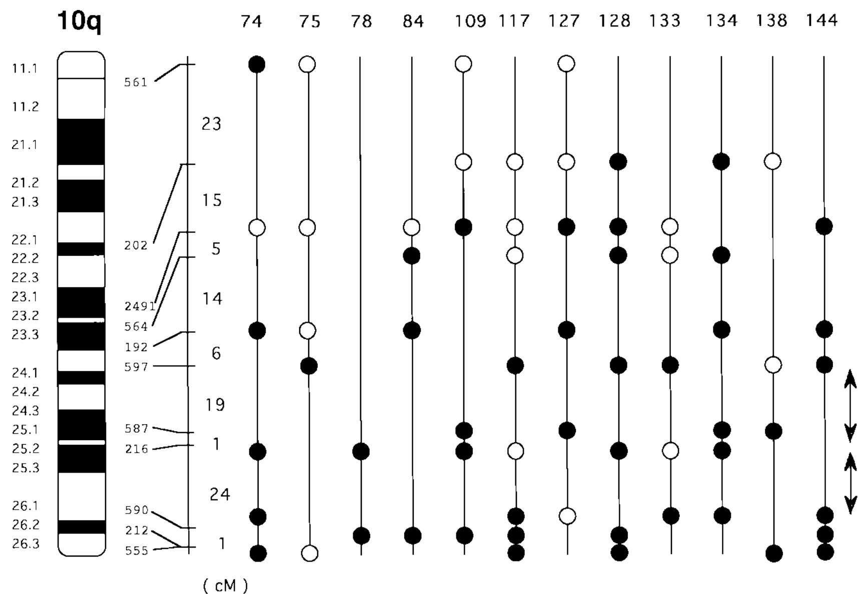

Fig. 2. Deletion map of chromosome 10q in 12 HCC tumors showing LOH at different loci. Tumor biopsy numbers are shown above and names of markers on the left. A diagram of chromosome 10q is at the far left with locations and order of microsatellite markers indicated; distances between loci indicated are in centimorgans (cM). Open circles, retention of heterozygosity; solid circles, LOH; gaps between circles, uninformativeness for specific markers. The commonly deleted regions are indicated by arrows at the right.

Clinicopathological data were available for 16 out of 37 HCC tumors. With respect to the grade of tumor differentiation, no LOH was detected in five moderately differentiated tumors, whereas six out of 11 poorly differentiated or undifferentiated HCC tumors showed LOH at loci on 10q.

Mutation analysis of PTEN/MMAC1 in HCC To examine whether *PTEN/MMAC1* is a candidate tumor suppressor gene for HCC, all tumors showing LOH at any locus on chromosome 10q were subjected to mutation analysis of the *PTEN/MMAC1* gene by SSCP and direct sequencing. One (tumor biopsy #134) out of 12 tumors showed an abnormally shifted band on SSCP analysis of exon 2. A somatic missense mutation (Met→Val) was detected at codon 35 in DNA sequence analysis of this tumor (Fig. 3, a and b).

DISCUSSION

We have presented here a detailed LOH analysis of chromosome 10q in 37 HCC tumors. Frequent LOHs (33%) were detected especially in the distal part of 10q at loci D10S212 and D10S590. Furthermore, analysis of deletion mapping defined two commonly deleted regions within the distal part of 10q (10q24-26). Recent studies have shown that the distal region of chromosome 10q is commonly deleted in glioma, lung cancer, melanoma, prostate cancer, bladder cancer, and thyroid tumor.[12, 15, 16, 20, 37] These results indicate that there are putative tumor suppressor gene(s) on the distal region of 10q, which are involved in various types of carcinomas.

Recent studies have isolated a putative tumor suppressor gene, *PTEN/MMAC1*, from near the centromeric region of 10q (10q23.3). The *PTEN/MMAC1*-encoded protein exhibits a lipid phosphatase activity, which is thought to be responsible for its tumor suppressor function.[38–40] Somatic mutations of this gene have been reported in various type of human cancers. The above findings suggest that there are plural tumor suppressor genes on chromosome 10q. To examine further the involvement of the *PTEN/MMAC1* gene in HCCs, we searched for mutations in 12 tumors showing LOH at loci on 10q. SSCP and mutation analysis detected a single somatic mutation (Met→Val) in exon 2 of *PTEN/MMAC1* gene, in advanced stage HCC tumor (Tumor Stage III, undifferentiated type). According to LOH analyses using a panel of eleven microsatellite markers, although allelic status was not informative at the locus of *PTEN/MMAC1* (D10S2491), tumor biopsy #134 showed LOH at all loci of flanking informative markers. These results suggest a loss of one allele at the *PTEN/MMAC1* locus, and that a mutation of this gene in the remaining allele occurred in the tumor DNA, resulting in inactivation of both alleles. Although the function of the mutated PTEN protein is not yet clear, a point mutation at codon 35 was previously reported in juvenile polyposis coli and was not observed in 93 controls.[41] These facts indicate that the variant may be a tumor-associated change. In the present study, only four out of 26 informative cases (15%) showed LOH at D10S2491, isolated from the *PTEN/MMAC1* locus, and the most frequent LOH was observed at the more distal part of 10q. These results indicated that plural tumor suppressor genes involved in hepatocarcinogenesis might exist on chromosome 10q.

In conclusion, we have obtained important new information on LOH in chromosome 10q and mutation of a putative tumor suppressor gene, *PTEN/MMAC1*, in a subset of HCC. Inactivation of *PTEN/MMAC1* and unknown tumor suppressor gene(s), present in the distal region of 10q (10q24-q26), may play an important role in the progression of HCC.

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REFERENCES

1) Murakami, Y., Hayashi, K., Hirohashi, S. and Sekiya, T. Aberrations of the tumor suppressor *p53* and retinoblastoma genes in human hepatocellular carcinomas. *Cancer Res.*, **51**, 5520–5525 (1991).
2) Zhang, X., Xu, H. J., Murakami, Y., Sachse, R., Yashima, K., Hirohashi, S., Hu, S. X., Benedict, W. F. and Sekiya, T. Deletions of chromosome 13q, mutations in retinoblastoma 1, and retinoblastoma protein state in human hepatocellular carcinoma. *Cancer Res.*, **54**, 4177–4182 (1994).
3) Yeh, S. H., Chen, P. J., Chen, H. L., Lai, M. Y., Wang, C. C. and Chen, D. S. Frequent genetic alterations at the distal region of chromosome 1p in human hepatocellular carcinomas. *Cancer Res.*, **54**, 4188–4192 (1994).
4) Wang, H. P. and Rogler, C. E. Deletions in human chromosome arms 11p and 13q in primary hepatocellular carcinomas. *Cytogenet. Cell Genet.*, **48**, 72–78 (1988).
5) Emi, M., Fujiwara, Y., Ohata, H., Tsuda, H., Hirohashi, S., Koike, M., Miyaki, M., Monden, M. and Nakamura, Y. Allelic loss at chromosome band 8p21.3-p22 is associated with progression of hepatocellular carcinoma. *Genes Chromosom. Cancer*, **7**, 152–157 (1993).
6) Takahashi, K., Kudo, J., Ishibashi, H., Hirata, Y. and Niho, Y. Frequent loss of heterozygosity on chromosome 22 in hepatocellular carcinoma. *Hepatology*, **17**, 794–799

(1993).
7) Nagai, H., Pineau, P., Tiollais, P., Buendia, M. A. and Dejean, A. Comprehensive allelotyping of human hepatocellular carcinoma. *Oncogene*, **14**, 2927–2933 (1997).
8) Piao, Z., Park, C., Park, J. H. and Kim, H. Deletion mapping of chromosome 4q in hepatocellular carcinoma. *Int. J. Cancer*, **79**, 356–360 (1998).
9) Ding, S. F., Habib, N. A., Dooley, J., Wood, C., Bowles, L. and Delhanty, J. D. Loss of constitutional heterozygosity on chromosome 5q in hepatocellular carcinoma without cirrhosis. *Br. J. Cancer*, **64**, 1083–1087 (1991).
10) Tsuda, H., Hirohashi, S., Shimosato, Y., Ino, Y., Yoshida, T. and Terada, M. Low incidence of point mutation of c-Ki-*ras* and N-*ras* oncogenes in human hepatocellular carcinoma. *Jpn. J. Cancer Res.*, **80**, 196–199 (1989).
11) Miyoshi, Y., Iwao, K., Nagasawa, Y., Aihara, T., Sasaki, Y., Imaoka, S., Murata, M., Shimano, T. and Nakamura, Y. Activation of the *beta-catenin* gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. *Cancer Res.*, **58**, 2524–2527 (1998).
12) Petersen, S., Rudolf, J., Bockmuhl, U., Gellert, K., Wolf, G., Dietel, M. and Petersen, I. Distinct regions of allelic imbalance on chromosome 10q22-q26 in squamous cell carcinomas of the lung. *Oncogene*, **17**, 449–454 (1998).
13) Simon, M., von, Deimling, A., Larson, J. J., Wellenreuther, R., Kaskel, P., Waha, A., Warnick, R. E., Tew, J. J. and Menon, A. G. Allelic losses on chromosomes 14, 10, and 1 in atypical and malignant meningiomas: a genetic model of meningioma progression. *Cancer Res.*, **55**, 4696–4701 (1995).
14) Sonoda, Y., Murakami, Y., Tominaga, T., Kayama, T., Yoshimoto, T. and Sekiya, T. Deletion mapping of chromosome 10 in human glioma. *Jpn. J. Cancer Res.*, **87**, 363–367 (1996).
15) Speaks, S. L., Sanger, W. G., Masih, A. S., Harrington, D. S., Hess, M. and Armitage, J. O. Recurrent abnormalities of chromosome bands 10q23-25 in non-Hodgkin's lymphoma. *Genes Chromosom. Cancer*, **5**, 239–243 (1992).
16) Zedenius, J., Wallin, G., Svensson, A., Bovee, J., Hoog, A., Backdahl, M. and Larsson, C. Deletions of the long arm of chromosome 10 in progression of follicular thyroid tumors. *Hum. Genet.*, **97**, 299–303 (1996).
17) Bose, S., Wang, S. I., Terry, M. B., Hibshoosh, H. and Parsons, R. Allelic loss of chromosome 10q23 is associated with tumor progression in breast carcinomas. *Oncogene*, **17**, 123–127 (1998).
18) Cappellen, D., Gil, D. D. M. S., Chopin, D., Thiery, J. P. and Radvanyi, F. Frequent loss of heterozygosity on chromosome 10q in muscle-invasive transitional cell carcinomas of the bladder. *Oncogene*, **14**, 3059–3066 (1997).
19) 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).
20) Isshiki, K., Elder, D. E., Guerry, D. and Linnenbach, A. J. Chromosome 10 allelic loss in malignant melanoma. *Genes Chromosom. Cancer*, **8**, 178–184 (1993).
21) Jones, M. H., Koi, S., Fujimoto, I., Hasumi, K., Kato, K. and Nakamura, Y. Allelotype of uterine cancer by analysis of RFLP and microsatellite polymorphisms: frequent loss of heterozygosity on chromosome arms 3p, 9q, 10q, and 17p. *Genes Chromosom. Cancer*, **9**, 119–123 (1994).
22) 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).
23) Cairns, P., Okami, K., Halachmi, S., Halachmi, N., Esteller, M., Herman, J. G., Jen, J., Isaacs, W. B., Bova, G. S. and Sidransky, D. Frequent inactivation of *PTEN/MMAC1* in primary prostate cancer. *Cancer Res.*, **57**, 4997–5000 (1997).
24) Cairns, P., Evron, E., Okami, K., Halachmi, N., Esteller, M., Herman, J. G., Bose, S., Wang, S. I., Parsons, R. and Sidransky, D. Point mutation and homozygous deletion of *PTEN/MMAC1* in primary bladder cancers. *Oncogene*, **16**, 3215–3218 (1998).
25) Chiariello, E., Roz, L., Albarosa, R., Magnani, I. and Finocchiaro, G. *PTEN/MMAC1* mutations in primary glioblastomas and short-term cultures of malignant gliomas. *Oncogene*, **16**, 541–545 (1998).
26) Kohno, T., Takahashi, M., Manda, R. and Yokota, J. Inactivation of the *PTEN/MMAC1/TEP1* gene in human lung cancers. *Genes Chromosom. Cancer*, **22**, 152–156 (1998).
27) 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*, **275**, 1943–1947 (1997).
28) Risinger, J. I., Hayes, A. K., Berchuck, A. and Barrett, J. C. *PTEN/MMAC1* mutations in endometrial cancers. *Cancer Res.*, **57**, 4736–4738 (1997).
29) Yokomizo, A., Tindall, D. J., Drabkin, H., Gemmill, R., Franklin, W., Yang, P., Sugio, K., Smith, D. I. and Liu, W. *PTEN/MMAC1* mutations identified in small cell, but not in non-small cell lung cancers. *Oncogene*, **17**, 475–479 (1998).
30) 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., 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).
31) Marsh, D. J., Dahia, P. L., Zheng, Z., Liaw, D., Parsons, R., Gorlin, R. J. and Eng, C. Germline mutations in *PTEN* are present in Bannayan-Zonana syndrome. *Nat. Genet.*, **16**, 333–334 (1997).
32) Marsh, D. J., Coulon, V., Lunetta, K. L., Rocca, S. P., Dahia, P. L., Zheng, Z., Liaw, D., Caron, S., Duboue, B.,

Lin, A. Y., Richardson, A. L., Bonnetblanc, J. M., Bressieux, J. M., Cabarrot, M. A., Chompret, A., Demange, L., Eeles, R. A., Yahanda, A. M., Fearon, E. R., Fricker, J. P., Gorlin, R. J., Hodgson, S. V., Huson, S., Lacombe, D., LePrat, F., Odent, S., Toulouse, C., Olopade, O. I., Sobol, H., Tishler, S., Woods, C. G., Robinson, B. G., Weber, H. C., Parsons, R., Peacocke, M., Longy, M. and Eng, C. Mutation spectrum and genotype-phenotype analyses in Cowden disease and Bannayan-Zonana syndrome, two hamartoma syndromes with germline *PTEN* mutation. *Hum. Mol. Genet.*, **7**, 507–515 (1998).

33) Japanese Liver Cancer Society. "The General Rules for Clinical and Pathological Study of Primary Liver Cancer," 3rd Ed., pp. 22–23 (1992). Kanehara Co., Tokyo.
34) Sato, T., Tanigami, A., Yamakawa, K., Akiyama, F., Kasumi, F., Sakamoto, G. and Nakamura, Y. Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer. *Cancer Res.*, **50**, 7184–7189 (1990).
35) Gyapay, G., Morissette, J., Vignal, A., Dib, C., Fizames, C., Millasseau, P., Marc, S., Bernardi, G., Lathrop, M. and Weissenbach, J. The 1993–94 Genethon human genetic linkage map. *Nat. Genet.*, **7**, 246–339 (1994).
36) 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).
37) Maier, D., Zhang, Z., Taylor, E., Hamou, M. F., Gratzl, O., Van, M. E., Scott, R. J. and Merlo, A. Somatic deletion mapping on chromosome 10 and sequence analysis of *PTEN/MMAC1* point to the 10q25-26 region as the primary target in low-grade and high-grade gliomas. *Oncogene*, **16**, 3331–3335 (1998).
38) Maehama, T. and Dixon, J. E. The tumor suppressor, *PTEN/MMAC1*, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J. Biol. Chem.*, **273**, 13375–13378 (1998).
39) Myers, M. P., Pass, I., Batty, I. H., Kaay, J. V., Stolarov, J. P., Hemmings, B. A., Wigler, M. H., Downes, C. P. and Tonks, N. K. The lipid phosphatase activity of *PTEN* is critical for its tumor suppressor function. *Proc. Natl. Acad. Sci. USA*, **95**, 13513–13518 (1998).
40) Tamura, M., Gu, J., Matsumoto, K., Aota, S., Parsons, R. and Yamada, K. M. Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. *Science*, **280**, 1614–1617 (1998).
41) Olschwang, S., Serova-Sinilnikova, O. M., Lenoir, G. M. and Thomas, G. PTEN germ-line mutations in juvenile polyposis coli. *Nat. Genet.*, **18**, 12–14 (1998).