[CANCER RESEARCH 57, 5221–5225, December 1, 1997]

Advances in Brief

MMAC1/PTEN Mutations in Primary Tumor Specimens and Tumor Cell Lines[1]

David H-F. Teng,[2] Rong Hu, Huai Lin, Thaylon Davis, Diana Iliev, Cheryl Frye, Brad Swedlund, Kipp L. Hansen, Vickie L. Vinson, Kathryn L. Gumpper, Lee Ellis, Adel El-Naggar, Marsha Frazier, Samar Jasser, Lauren A. Langford, Jeff Lee, Gordon B. Mills, Mark A. Pershouse, Raphael E. Pollack, Carmen Tornos, Patricia Troncoso, W. K. Alfred Yung, Gregory Fujii, Amy Berson, Robert Bookstein, Joseph B. Bolen, Sean V. Tavtigian, and Peter A. Steck

Myriad Genetics Inc. [D. H. F. T., R. H., T. D., D. I., C. F., B. S., S. V. T.] and Myriad Genetics Laboratories Inc. [K. L. H., V. L. V., K. L. G.], Salt Lake City, Utah 84108; Department of Neuro-Oncology [H. L., S. J., M. A. P., W. K. A. Y, P. A. S.], Division of Surgery [L. E., J. L., R. E. P.], Division of Medicine [M. F., G. B. M.], and Department of Pathology [A. E. N., L. A. L., C. T., P. T.], University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030; Department of Cellular Signaling, DNAX Research Institute, Palo Alto, California 94303 [G. F., A. B., J. B. B.]; and Canji, Inc., San Diego, California 92121 [R. B.]

Abstract

A candidate tumor suppressor gene, *MMAC1/PTEN*, located in human chromosome band 10q23, was recently identified based on sequence alterations observed in several glioma, breast, prostate, and kidney tumor specimens or cell lines. To further investigate the mutational profile of this gene in human cancers, we examined a large set of human tumor specimens and cancer cell lines of many types for 10q23 allelic losses and *MMAC1* sequence alterations. Loss of heterozygosity (LOH) at the *MMAC1* locus was observed in approximately one-half of the samples examined, consistent with the high frequency of 10q allelic loss reported for many cancers. Of 124 tumor specimens exhibiting LOH that have been screened for *MMAC1* alterations to date, we have detected variants in 13 (~10%) of these primary tumors; the highest frequency of variants was found in glioblastoma specimens (~23%). Novel alterations identified in this gene include a missense variant in a melanoma sample and a splicing variant and a nonsense mutation in pediatric glioblastomas. Of 76 tumor cell lines prescreened for probable LOH, microsequence alterations of *MMAC1* were detected in 12 (~16%) of the lines, including those derived from astrocytoma, leukemia, and melanoma tumors, as well as bladder, breast, lung, prostate, submaxillary gland, and testis carcinomas. In addition, in this set of tumor cell lines, we detected 11 (~14%) homozygous deletions that eliminated coding portions of *MMAC1*, a class of abnormality not detected by our methods in primary tumors. These data support the occurrence of inactivating *MMAC1* alterations in multiple human cancer types. In addition, we report the discovery of a putative pseudogene of *MMAC1* localized on chromosome 9.

Introduction

Of the two genetic events required to mutationally inactivate tumor suppressor genes, the first may be either inherited as a germ-line lesion or acquired as a somatic mutation. The second event typically consists of complete or partial loss of the chromosome carrying the remaining wild-type allele, which is observed as LOH[3] of nearby polymorphic markers. Alternatively, both alleles of the tumor suppressor gene may be inactivated by two independent, localized mutations. Homozygous deletion (complete loss of genetic material) is the pathognomonic type of tumor suppressor mutation. Through the identification of homozygous deletions in tumor cell lines, Steck *et al.* (1) and Li *et al.* (2) discovered a human candidate tumor suppressor gene, *MMAC1/PTEN*, localized on 10q23, that encodes a protein phosphatase that has been reported to function with dual specificity *in vitro* (3). In parallel, in a search for novel phosphatases, Li and Sun (4) identified the *TEP1* gene, which encoded a M_r ~55,000 protein that exhibited tyrosine phosphatase activity (4). Sequence comparisons showed that *TEP1* was identical to *MMAC1/PTEN*. Initial screens revealed that the *MMAC1* gene was mutated in tumor cell lines from brain, prostate, and breast cancers, as well as primary tumor specimens from GBMs and breast and kidney carcinomas (1, 2). Furthermore, germ-line *MMAC1* mutations are apparently responsible for a subpopulation of individuals that suffer from Cowden's disease (5–7), a rare familial syndrome that confers a high risk of breast cancer, and a related harmartomatous polyposis syndrome, Bannayan-Zonana syndrome (8).

In this study, we investigated the occurrence of alterations to the *MMAC1* gene in a series of human TCLs and tumor specimens derived from numerous kinds of cancers. Mutation analysis was performed by sequencing the nine exons and adjacent intronic splice-junction regions of *MMAC1*. This strategy allowed us to assess the status of the coding and splice-junction sequences of this gene.

Materials and Methods

Tumor Specimens and Tumor Cell Lines. Tumor specimens, which were comprised predominantly of tumor cells, were designated and obtained from pathologists at M. D. Anderson Cancer Center. Total genomic DNA was purified from frozen specimens or deparaffinized sections as described previously (9). Based on the results of the LOH analyses and by comparison of mutant to wild-type signals observed in the sequence data of tumor specimens, we estimated that many of the samples examined contained 10–40% normal cell contamination. Total genomic DNA was purified from cancer cell lines using the Easy-DNA kit (Invitrogen). Approximately 3–20 ng of DNA was used as template in the PCR amplifications described below.

LOH Analysis. LOH analysis was performed as described previously (10, 11). The polymorphic short tandem repeat markers used in this study were: *D10S1687* (H.I., 0.81; Location Database (12) radiation map position from p-telomere, R.L., 85 Mb), *D10S579* (H.I., 0.59; R.L., 86.4 Mb), *D10S541* (H.I., 0.78; R.L., 86.5 Mb), *AFM280WE1* (H.I., not determined; R.L., 87 Mb), *AFMA114XB1* (H.I., 0.70; R.L., 91.9 Mb), and *D10S1753* (H.I., 0.74; R.L., 92.48 Mb). The *MMAC1* locus as defined by *AFM086WE1* is at about 86.5 Mb. LOH was assessed in primary tumor specimens, in the majority of cases, by quantitatively comparing polymorphic marker amplicons generated from tumor and normal DNAs of each individual tested. In the case of TCLs and some primary tumors, LOH was assessed on the basis of combined apparent hemizygosity of *AFMA114XB1*, *D10S541*, and *D10S1753*; the likelihood that all three of these markers are homozygous in a given sample is less than 0.017.

Received 7/16/97; accepted 10/17/97.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work is supported in part by NIH Grants CA56041 and CA55261 (to P. A. S.) and the generous support of the Pediatric Brain Tumor Foundation (to P. A. S., L. A. L.) and The Gilland Foundation (to W. K. A. Y.). The DNAX Research Institute is supported by Schering-Plough Corporation.

[2] To whom requests for reprints should be addressed, at Myriad Genetic, Inc., 390 Wakara Way, Salt Lake City, UT 84108. Phone: (801) 584-3676; Fax: (801) 584-3650; E-mail: tengd@myriad.com.

[3] The abbreviations used are: LOH, loss of heterozygosity; GBM, glioblastoma multiforme; TCL, tumor cell line; H.I., heterozygosity index; R.L., radiation map location.

Homozygous Deletion Screen. Using the cell line genomic DNAs as templates, nested PCR amplifications were performed with either TaqPlus (Stratagene) or AmpliTaq Gold (Perkin-Elmer). The primers used for generating *MMAC1* and *MKK4* amplicons and the PCR conditions used are as described below and by Teng *et al.* (13). Twenty μl of the secondary reactions were fractionated on 2–3% Nu Sieve (FMC Bioproducts) agarose gels and subsequently visualized.

Mutation Screen. We performed nested PCR amplifications on genomic DNAs of tumor specimens or TCLs and screened the resulting amplicons for *MMAC1* sequence variants according to the procedures of Steck *et al.* (1) with the several modifications: (*a*) exon 6 was screened with a single secondary amplicon amplified using the exon 6 FB-RR primer pair; (*b*) after a primary amplification of exon 8 using FA-RP primers, the exon was screened as two secondary amplicons using the following FB-RQ and FC-RR primers: CA6.ex8.FB, GTTTTCCCAGTCACGACGAGGTGACAGATTTTCTTTTTTA; CA6.ex8.RQ, AGGAAACAGCTATGACCATTCGGTTGGCTTTGTCTTTA; CA6.ex8.FC, GTTTTCCCAGTCACGACGCATTTGCAGTATAGAGCGT; and CA6.ex8.RR, AGGAAACAGCTATGACCATAGCTGTACTCCTAGAATTA; and (*c*) because mononucleotide runs in certain introns caused poor dye-primer sequencing, we obtained dye-terminator sequence data on secondary amplicons exon 8 FB-RQ and exon 9 FB-RR using the nested primers TTTTTTTTTAGGACAAAATGTTTC and AATTCAGACTTTTGTAATTTGTG, respectively. We obtained greater than 95% double-stranded coverage of the *MMAC1* coding sequence for all samples screened; all mutations were confirmed by sequencing a newly amplified product.

Characterization of a Putative *MMAC1* Pseudogene. DNA fragments were amplified from a human fetal brain cDNA library using Pfu polymerase and a nested PCR strategy. The primer pair used in the first round of amplification was CTTCAGCCACAGGCTCCCAGAC and GGTGTTTTATCCCTCTTG, after which the reaction was diluted 20-fold and reamplified with CGGGATCCATGACAGCCATCATCAAAGAGATC and CGGAATTCTCAGACTTTTGTAATTG primers. The PCR conditions used were an initial denaturation step at 94°C for 5 min, followed by 30 cycles of 94°C for 45 s, 55°C for 30 s, and 72°C for 1 min. The amplified products were cloned and then sequenced.

To determine the chromosomal location of this pseudogene, we performed radiation hybrid mapping using the Genebridge 4 panel (Genome Systems) and the following primer pair designed to generate a specific 303-bp product from the pseudogene but not *MMAC1*: ATCCTCAGTTTGTGGTCTGC and TTGTCATTATCTGCACGCTC. Using this sequence tagged site, we determined that the pseudogene was located at about 160 cR on chromosome 9; Southern blotting on monochromosomal blots supported the genomic location. Additionally, we have isolated two bacterial artificial chromosome clones, 145c22 and 188l22, that contain this pseudogene and have obtained sequence directly from bacterial artificial chromosome 188l22. Comparison of *MMAC1* coding sequence to that of the pseudogene revealed the following base differences: T2G, C89T, T202C, T242C, G248A, A258G, G397A, A405T, G407A, T531C, T544G, C556G, A672G, C700T, A705G, C720T, C900T, and A942G. The sequence of this putative pseudogene has been submitted to GenBank (accession number AF023139).

Results

Because the *MMAC1* gene encodes a candidate tumor suppressor, our initial step toward identifying new mutations in this gene was to prescreen primary tumors and TCLs for LOH within this region of 10q23. Altogether, 246 primary tumor specimens and 165 TCLs were examined using polymorphic short tandem repeat markers on chromosome 10 located near the *MMAC1* locus (Table 1). In this panel of samples, we observed LOH in primary tumor specimens at frequencies ranging from 20% in colon specimens to 75% in GBMs, with an overall frequency of ~49%. For TCLs with sample sizes greater than nine, the incidence of LOH varied from 28% (colon) to 82% (GBMs), with an overall frequency of ~46%.

To search for coding variants of *MMAC1* in primary tumors, we sequenced amplicons consisting of the exons and flanking splice junctions of this gene amplified from tumor DNAs that displayed LOH. This approach, although highly reliable and sensitive for detecting coding and splicing mutations, is limited by its difficulty in the detection of: (*a*) *MMAC1* mutations in tumors without LOH; (*b*) mutations in regulatory regions of the gene; and (*c*) partial or complete homozygous deletions. In the latter case, the absence of amplification from tumor DNA will be masked by amplification of DNA from contaminating normal cells with wild-type sequence or tumor

Table 1 *LOH analyses of tumor specimens and tumor cell lines*

	Tumor specimens			Tumor cell lines			
Tumor type	LOH/screened[a]	Analyzed[b]	Variants[c,d]	LOH/screened[a]	Analyzed[b]	Variants[c]	*MMAC1* HDs[c]
Brain (gliomas)	40/53[d] (75%)	26[d]	2fs, 1n, 1id, 2m	9/11 (82%)	9	1fs, 1n, 2m	3
Pediatric brain	5/7	5	1n, 1sp				
Bladder				3/4	3	0	1
Breast	32/67[d] (48%)	31[d]	2fs, 1id	14/22 (64%)	14	1fs, 2m	1
Colon	3/15[e] (20%)	1	0	7/25 (28%)	7	0	0
Head and neck	9/14 (64%)	9	0				
Kidney	8/20[d] (40%)	8[d]	1fs				
Leukemia				11/23 (48%)	11	2m	0
Lung	10/27 (37%)	7	0	7/17 (41%)	7	0	1
Melanoma	10/21[d] (48%)	10[d]	1m	7/14 (50%)	7	0	4
Ovarian	10/19 (52%)	9	0	3/8	3	0	0
Pancreatic	7/19 (37%)	0		5/12 (42%)	5	0	0
Prostate	10/24 (42%)	6	0	2/2[f]	2	1fs	1
Sarcomas	4/16 (25%)	4	0				
Submaxillary gland				1/1	1	1m	0
Testis				3/5	3	1m	0
Thyroid	6/17 (35%)	2	0	0/2			
Metastatic[g]	6/10 (60%)	6	0				
Other				4/19[h]	4	0	0
Total	160/329[d] (49%)	124[d,i]	5fs, 2n, 2id, 3m, 1sp	76/165[d] (46%)	76[d]	3fs, 1n, 8m	11 HDs

[a] LOH percentage was only calculated for sample sizes greater than nine.

[b] Samples with apparent LOH that amplified and sequenced successfully (>95% coding sequence screened). Some of the primary tumor DNAs were isolated from microdissected paraffin-embedded sections and failed to amplify or sequence at >95% coverage due to poor template quality.

[c] Summary of the *MMAC1* variants detected; HD, homozygous deletions; fs, frameshifts; id, in-frame deletions; n, nonsense; m, missense; sp, splicing.

[d] These totals include all of the glioma, breast, kidney, and melanoma primary tumor samples that were reported previously by Steck *et al.* (1).

[e] Five of these colon samples consisted of cancers that had metastasized to the liver, although the liver metastases exhibited no LOH.

[f] These two prostate lines, NCIH660 (TCL10F4) and LNCAP, were characterized previously (1, 2).

[g] These metastatic tumor specimens originated from adenocarcinomas, a sarcoma, a renal cell carcinoma, and a melanoma. The metastatic lesions were to the lung, except the melanoma which was to the groin.

[h] These 19 TCLs were derived from 2 lymphomas, 3 neuroblastomas, 2 retinoblastomas, 6 cecum, 1 duodenum, and 4 uterine carcinomas.

[i] Of these 124 specimens analyzed by sequencing, 45 have been reported (1).

Table 2 *MMAC1 variants identified in primary tumors and tumor cell lines*

Sample	Type	Mutation	Exon/Intron	Codon	Predicted effect
PGT-2	Pediatric glioma[a]	G → T at −1	Intron 2		Splicing variant
MT-1	Melanoma[a]	CC112-113TT	Exon 2	38	Pro → Phe
TCL10B1	Breast	T323G	Exon 5	108	Leu → Arg
TCL10H2	Leukemia	T331C	Exon 5	111	Trp → Arg
TCL11E12	Glioblastoma	T335G	Exon 5	112	Leu → Arg
PGT-5	Pediatric glioma[a]	C388T	Exon 5	130	Arg → Stop
TCL10A7	Breast	G407A	Exon 5	136	Cys → Tyr
TCL10F5	Submaxillary gland	T455C	Exon 5	152	Leu → Pro
TCL10H8	Leukemia	C517T	Exon 6	173	Arg → Cys
TCL10F7	Testis	G518C	Exon 6	173	Arg → Pro
TCL11F5	Glioblastoma	C697T	Exon 7	233	Arg → Stop
BT-88	Breast[a,c]	705 del A	Exon 7	235	Protein truncation
TCL10A3	Breast[b]	823 del G	Exon 7	275	Protein truncation

[a] Primary tumor specimens.

[b] This cell line, TCL10A3, is BT549, and its mutation was reported by Li *et al.* (2). The *MMAC1* alterations observed in three other TCLs, glioma T98G, glioma U373, and prostate LNCAP, have been reported by Steck *et al.* (1) and were not included in this table. These previously characterized cell lines were part of the panel of 165 TCLs that were selected in an unbiased manner for *MMAC1* alterations and should, therefore, generate variant frequencies representative of TCLs examined for the given types of cancers.

[c] Analysis of corresponding normal DNA has shown that the *MMAC1* mutation of this primary breast tumor sample is somatic. Similar analysis of the *MMAC1* alterations in the other three primary tumor specimens was not possible because corresponding normal DNAs were not available. We have, however, determined that all nine primary tumor mutations reported previously by Steck *et al.* (1) arose somatically.

cells that do not exhibit deletion of the gene. Previously, we reported that the incidence of *MMAC1* coding variants in glioblastomas and breast and kidney carcinomas were 6 of 26, 2 of 14, and 1 of 4, respectively (1). In this study, of 79 tumor specimens displaying LOH, we detected a frameshift mutation in 1 of 17 breast carcinomas, a missense variant in 1 of 10 melanomas, and a nonsense mutation and a splicing variant in 2 of 5 pediatric GBMs (Table 2).

In addition to primary tumors, we examined a set of TCLs for alterations in the *MMAC1* gene. These TCLs permitted us to investigate cancer types that were not represented in the panel of primary tumors screened, including leukemia, lymphoma, neuroblastoma, retinoblastoma, as well as bladder, testis, and uterine cancers. Of the 76 TCLs exhibiting LOH, we detected 11 homozygous deletions that affected the coding regions of *MMAC1* (Fig. 1). The homozygous deletions were present in TCLs from astrocytomas (1 of 1), bladder carcinoma (1 of 3), breast carcinoma (1 of 14), glioblastoma (1 of 8), lung carcinoma (1 of 7), melanoma (4 of 7), and prostate carcinoma (1 of 2). Whereas two of the cell lines had lost all nine *MMAC1* exons, the other nine TCLs had homozygously deleted different coding portions of the gene. Sequence analysis of the remaining 65 TCLs revealed three frameshifts and one nonsense and eight nonconservative missense variants (Table 2).

One potential complication of future studies on *MMAC1* is the presence of a putative *MMAC1* pseudogene in the human genome. In

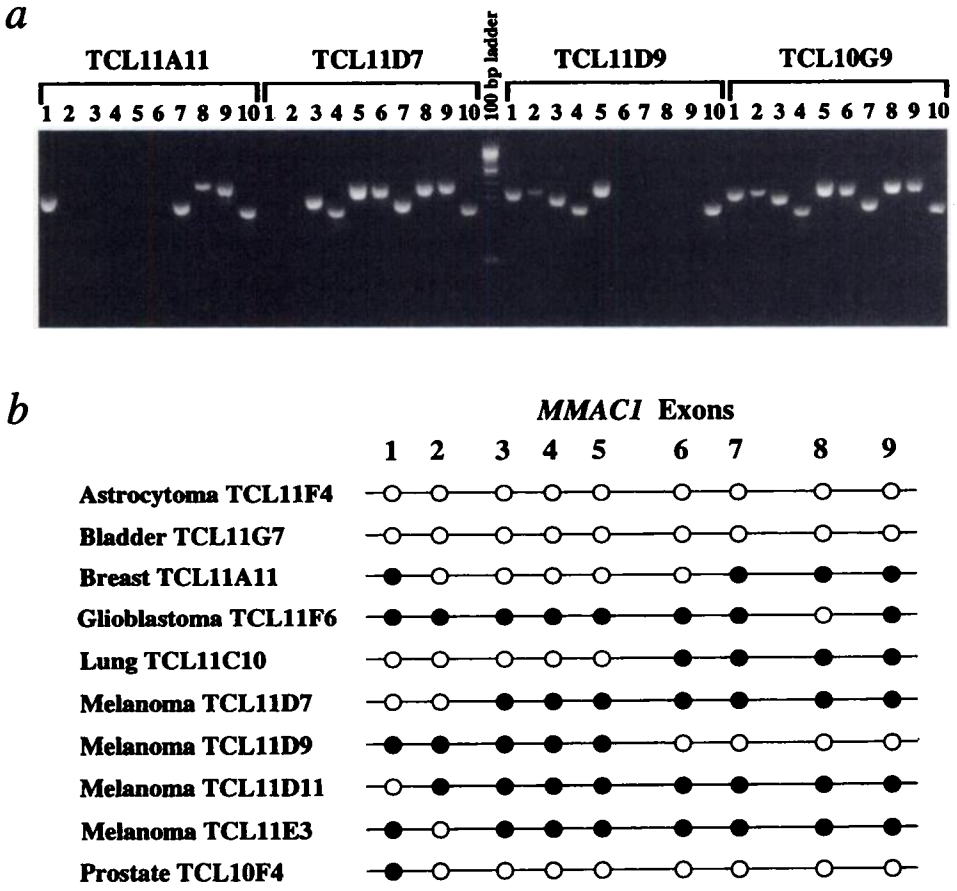

Fig. 1. Homozygous deletions of the *MMAC1* gene in human tumor cell lines. *a*, four cell lines: breast carcinoma TCL11A11, melanoma TCL11D7, melanoma TCL11D9, and leukemia TCL10G9 (control line with wildtype *MMAC1*), each examined by PCR amplification using the following sequence tagged sites: *1*, *MMAC1* exon 1; *2*, *MMAC1* exon 2; *3*, *MMAC1* exon 3; *4*, *MMAC1* exon 4; *5*, *MMAC1* exon 5; *6*, *MMAC1* exon 6; *7*, *MMAC1* exon 7; *8*, *MMAC1* exon 8; *9*, *MMAC1* exon 9; and *10*, control *MKK4* exon 8. *b*, homozygous deletions observed in the *MMAC1* gene of TCLs screened. ●, exons that are not homozygously deleted; ○, exons that are lost. One of the glioblastoma TCLs, A172, with a homozygous deletion affecting *MMAC1* was reported previously (1, 2) and was, therefore, not included in this schematic.

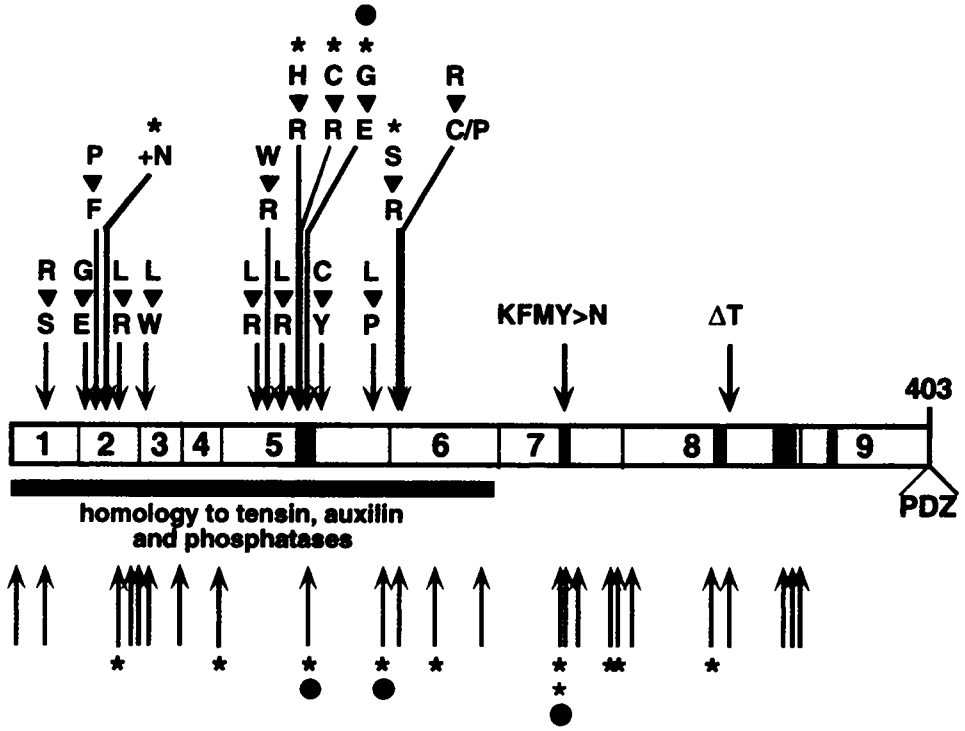

Fig. 2. Representation of the putative functional domains of MMAC1 and the locations of identified alterations. The NH_2-terminal half of MMAC1 is homologous to phosphatases, as well as the cytoskeletal proteins, tensin and auxilin. Also shown are the locations of the core phosphatase domain (*red box*), three potential tyrosine phosphorylation sites (*blue boxes*), and two potential serine phosphorylation sites (*yellow boxes*). The PDZ motif, ITKV, is located at the COOH terminus of the protein. Shown are the *MMAC1* alterations detected in this study, as well as many of the other variants identified to date (*1, 2,* and *5–8*); *blue arrows*, missense substitutions; *black arrows*, in-frame insertions or deletions; *green arrows*, potential splicing variants; *red arrows*, frameshift or nonsense mutations that result in MMAC1 truncations. Homozygous deletions of *MMAC1* are not included in this compilation. *Black asterisks*, germ-line mutations that were detected in Cowden's patients (*5–7*); *red asterisks*, two germ-line lesions found in individuals with Bannayan-Zonana syndrome (*8*). ●, lesions that have been observed in two or more presumably independent DNA samples. A large sampling of individuals, however, has not yet been performed to statistically determine the frequency at which many of these genetic alterations are present in the unaffected population.

the process of generating a *MMAC1* expression construct from cDNAs, we found that several independent clones harbored numerous sequence alterations when compared to wild-type *MMAC1*. Control PCR experiments then showed that certain primers designed from the coding sequences of *MMAC1* were able to amplify a product from human total genomic DNA. Sequence analysis of the amplified fragment revealed that this putative pseudogene was spliced before integration into genomic DNA, and that it differs from the coding sequence of *MMAC1* in 18 of 1209 bases (see "Materials and Methods"). One of the sequence differences is the T2G change of this pseudogene, which eliminates the equivalent predicted initiation codon of *MMAC1*. Using a sequence tagged-site that specifically amplified a product from the pseudogene but not *MMAC1*, we performed radiation hybrid mapping and determined that the pseudogene was located at about 160 cR on chromosome 9. Reverse transcription-PCR analyses suggest that this putative pseudogene is expressed in several cell lines, with or without the expression of wild-type *MMAC1*.[4]

Discussion

We have investigated a large panel of tumors and TCLs, prescreened for LOH, for alterations in *MMAC1*. In this set of 79 primary tumors, we detected four inactivating *MMAC1* mutations. Taken together with our previous findings (1), 8 of 31 glioblastomas (26%), 3 of 31 breast (10%), 1 of 8 kidney (13%), and 1 of 11 melanoma (9%) primary tumors showed *MMAC1* alterations; this corresponds to an overall variant frequency of ~10%. Of interest, two of the five pediatric GBMs exhibited *MMAC1* alterations that should lead to the expression of nonfunctional protein (Table 2), suggesting that further analysis of *MMAC1* involvement in this childhood disease is warranted. In addition, the missense variant observed in the primary melanoma sample was a consequence of a CC112–113TT change, an alteration that is commonly found in skin cancers harboring genes mutated by UV radiation (14, 15). In a set of 76 TCLs, we observed 12 potential inactivating *MMAC1* micromutations, corresponding to a variant frequency of ~16%. Statistical analysis provides no evidence for a difference in the frequency of *MMAC1* variants (excluding those homozygously deleted) observed between tumor specimens and cell lines for each cancer type examined in Table 1.

In the set of TCLs examined, we observed 11 lines with homozygous deletions eliminating coding portions of the gene. If homozygous deletions of *MMAC1* occur in primary tumors, it seems plausible that their frequency should mirror that observed in TCLs. Although about one-half of the TCLs that harbor alterations in *MMAC1* consist of homozygous deletions, no homozygous deletions of this gene have been reported in primary tumor specimens to date. This discrepancy is likely due to the inability of many screening methods of detecting this kind of lesion in primary tumors that are invariably heterogeneous and contaminated with normal cells. Indeed, based on the signals observed in the LOH analyses and mutation screening sequence data, we estimated that most of the tumor specimens that we examined contained 10–40% normal cell contamination. In control experiments, we have determined that even the presence of 5% contaminating normal DNA within tumor samples will prevent the identification of homozygous deletions using our procedures. Three additional observations suggest that only the more malignant cancer cells within a heterogeneous tumor may harbor mutations in *MMAC1*: (*a*) mutations of *MMAC1* are predominantly observed in GBMs but not in lower grade astrocytomas, suggesting that alteration to the gene represents a late cancer progression event (1); (*b*) allelic deletions to chromosome 10 have been observed predominantly in the advanced or malignant forms of meningiomas, melanomas, and prostate carcinomas (16–18); and (*c*) the insertion of a functional chromosome 10 into rat prostate carcinoma cells inhibited their metastatic capabilities (19). Consequently, the heterogeneous composition of tumor specimens would further hinder the detection of homozygous deletions.

[4] G. Fujii and J. B. Bolen, unpublished observation.

Based on its sequence, *MMAC1* appears to encode a tyrosine phosphatase or dual-specificity phosphatase with homology to the cytoskeleton-associated proteins, chicken tensin and bovine auxilin (1, 2). Recent work by Myers *et al.* (3) reveals that MMAC1 can function as a dual-specificity phosphatase *in vitro*. The NH_2-terminal half of MMAC1 is homologous to several phosphatases, and its core phosphatase motif is present at residues 122–134 (20, 21). Thus, the NH_2-terminal region of MMAC1 may have enzymatic and cellular localization activities. The COOH-terminal portion of MMAC1 contains three potential tyrosine phosphorylation sites at residues 240, 315, and 336. If phosphorylated, tyrosine 315 would represent a potential SH2 binding site because there is a leucine residue located three residues COOH-terminal from the tyrosine (22). Two potential serine phosphorylation sites are also present within the COOH-terminal half of MMAC1. Serine residue 338 represents a potential Ca^{2+}/calmodulin-dependent protein kinase II site, whereas serine 355 represents a potential casein kinase II site (23). The last four COOH-terminal amino acids, ITKV, represent a potential PDZ binding domain (24, 25). PDZ domains are present in a variety of intracellular proteins and are thought to mediate protein-protein interactions by binding directly to the COOH-terminal ends of target proteins.

A compilation of *MMAC1* alterations shows that the spectrum of variants is diverse (Fig. 2). All of the nonconservative missense substitutions identified are found in the NH_2-terminal portion of MMAC1 within its phosphatase domain. In contrast, lesions that result in the truncation of MMAC1 are distributed throughout the gene. If all of the truncated forms of MMAC1 are nonfunctional, then the data indicate that the COOH-terminal region of MMAC1 is essential for the expression of active protein. This is consistent with the notion that the potential phosphorylation sites and PDZ motif are important for MMAC1 function. Alternatively, the sequences of the COOH-terminal region of this protein may be required for proper folding. Of interest, the only germ-line mutations in *MMAC1* reported to date have been detected in individuals with Cowden-like syndromes (5–8); all other primary tumor *MMAC1* variants characterized have arisen somatically (Table 2 and ref. 2).

The diversity of the *MMAC1* alterations predict that many distinct lesions of this gene exist in the population. Most of the deleterious mutations identified in *MMAC1* to date have been either coding or splice-junction variants. However, other potential mechanisms of inactivating MMAC1 should be investigated, including events that alter its level of expression. Indeed, Li and Sun (4) have reported that *MMAC1* can be down-regulated by TGF-β in human keratinocytes. Moreover, preliminary evidence in human gliomas suggests that *MMAC1* expression is significantly down-regulated in the higher grade tumors.[5] Our discovery of a putative *MMAC1* pseudogene indicates that future studies on *MMAC1*, particularly those investigating its message levels, will have to be designed to distinguish it from this pseudogene. The results reported in this study should provide a framework for future structure-function studies on MMAC1 and for elucidating its role in tumorigenesis. Overall, the data suggest that MMAC1 is a tumor suppressor that plays a role in the genesis of many types of cancers.

Acknowledgments

We are indebted to J. Mitchell, C. Atkinson, A. Bush, J. Chung, E. Gomez, P. Ha, A-M. Jensen, J. Palmatier, T. Le, S. Richards, H. Rojeski, C. Smith, P-S. Su, S. Terry, T. Tran, J. Varelman, and D. Woodland as well as other diagnostic people for providing excellent technical assistance. We thank Jeng-Horng Her and Alyssa Morimoto for helpful discussions.

[5] P. A. Steck, unpublished observation.

References

1. 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., Fyre, C., Hu, R., Swedlund, B., Teng, D. H. F., and Tavtigian, S. Identification of a candidate tumor suppressor gene, *MMAC1*, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat. Genet., *15:* 356–362, 1997.
2. 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., Hibhoosh, 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.
3. Myers, M. P., Stolarov, J. P., Eng, C., Li, J., Wang, S. I., Wigler, M. H., Parsons, R., and Tonks, N. K. P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. Proc. Natl. Acad. Sci. USA, *94:* 9052–9057, 1997.
4. 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.
5. Liaw, D., Marsh, D. J., Li, J., Dahia, P. L. M., Wang, S. I., Zheng, Z., Bose, S., Call, K. H., 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.
6. Nelen, M. R., van Staveren, W. C. G., Peeters, E. A. J., Hassel, M. B., Gorlin, R. J., Hamm, H., Lindboe, C. F., Fryns, J-P., Sijmons, R. H., Woods, D. G., Mariman, E. C. M., Padberg, G. W., and Kremer, H. Germline mutations in the *PTEN/MMAC1* gene in patients with Cowden disease. Hum. Mol. Genet., *6:* 1383–1387, 1997.
7. Tsou, H. C., Teng, D. H-F., Ping, X. L., Brancolini, V., Davis, T., Hu, R., Xie, X-X., Gruener, A. C., Schrager, C. A., Christiano, A. M., Eng, C., Steck, P., Ott, J., Tavtigian, S. V., and Peacocke, M. Role of *MMAC1* mutations in early onset breast cancer: causative in association with Cowden's syndrome and excluded in *BRCA1*-negative cases. Am. J. Hum. Genet., in press, 1997.
8. Marsh, D. J., Dahia, P. L. M., Zheng, Z., Liaw, D., Parsons, R., Gorlin, R. J., and Eng, C. Germline mutations in *PTEN* ar present in Bannayan-Zonana syndrome. Nat. Genet., *16:* 333–334, 1997.
9. Pershouse, M. A., El-Naggar, A. K., Hurr, K., Lin, H., Yung, W. K. A., and Steck, P. A. Deletion mapping of chromosome 4 in head and neck squamous cell carcinoma. Oncogene, *14:* 369–373, 1997.
10. Steck, P. A., Hadi, A., Cheong, H. C., Yung, W. K. A., and Pershouse, M. A. Evidence for two tumor suppressive loci on chromosome 10 involved in glioblastomas. Genes Chromosomes Cancer, *12:* 255–261, 1995.
11. Teng, D. H. F., Bogden, R., Mitchell, J., Baumgard, M., Bell, R., Berry, S., Davis, T., Ha, P. C., Kehrer, R., Jammulapati, S., Chen, Q., Offit, K., Skolnick, M. H., Tavtigian, S. V., Jhanwar, S., Swedlund, B., Wong, A. K. C., and Kamb, A. Low incidence of *BRCA2* mutation in breast carcinoma and other cancers. Nat. Genet., *13:* 241–248, 1996.
12. Collins, A., Frezal, J., Teague, J., and Morton, N. E. A metric map of humans: 23,500 loci in 850 bands. Proc. Natl. Acad. Sci. USA, *93:* 14771–14775, 1996.
13. Teng, D. H-F., Perry, W., Hogan, J. K., Baumgard, M., Bell, R., Berry, S., Davis, T., Frank, D., Frye, C., Hattier, T., Hu, R., Jammulapati, S., Janecki, T., Leavitt, A., Mitchell, J. T., Pero, R., Sexton, D., Schroeder, M., Su, P. H., Swedlund, B., Kyriakis, J., Avruch, J., Bartel, P., Wong, A. K. C., Oliphant, A., Thomas, A., Skolnick, M. H., and Tavtigian, S. V. Human MAP kinase kinase 4 as a candidate tumor suppressor. Cancer Res., *57:* 4177–4182, 1997.
14. Ananthaswamy, H. N., and Pierceall, W. E. Molecular mechanisms of ultraviolet radiation carcinogenesis. Photochem. Photobiol., *52:* 1119–1136, 1990.
15. Ziegler, A., Jonason, A., Simon, J., Leffell, D., and Brash, D. E. Tumor suppressor gene mutations and photocarcinogenesis. Photochem. Photobiol., *63:* 432–435, 1996.
16. Herbst, R. A., Weiss, J., Ehnis, A., Cavenee, W. K., and Arden, K. C. Loss of heterozygosity for 10q22–qter in malignant melanoma progression. Cancer Res., *54:* 3111–3114, 1994.
17. Ittmann, M. Allelic loss on chromosome 10 in prostate adenocarcinoma. Cancer Res., *56:* 2143–2147, 1996.
18. Rempel, S. A., Schwechheimer, K., Davis, R. L., Cavenee, W. K., and Rosenblum, M. L. Loss of heterozygosity for loci on chromosome 10 is associated with morphologically malignant meningioma progression. Cancer Res., *53:* 2386–2392, 1993.
19. Murakami, Y. S., Albertsen, H., Brothman, A. R., Leach, R. J., and White, R. L. Suppression of the malignant phenotype of human prostate cancer cell line PPC-1 by introduction of normal fragments of human chromosome 10. Cancer Res., *56:* 2157–2160, 1996.
20. Denu, J., Stuckey, J. A., Saper, D., and Dixon, J. E. Form and function in protein dephosphorylation. Cell, *87:* 361–364, 1996.
21. Tonks, N. K., and Neel, B. G. From form to function: signaling by protein tyrosine phosphatases. Cell, *87:* 365–368, 1996.
22. Songyang, Z., and Cantely, L. C. Recognition and specificity in protein tyrosine kinase mediated signalling. Trends Biochem. Sci., *20:* 470–475, 1995.
23. Hardie, G., and Hanks, S. The Protein Kinase Facts Book. Protein-Serine Kinases. London: Academic Press, 1995.
24. Fanning, A. S., and Anderson, J. M. Protein-protein interactions: PDZ domain networks. Curr. Biol., *6:* 1385–1388, 1996.
25. Saras, J., and Heldin, C-H. PDZ domains bind carboxy-terminal sequences of target proteins. Trends Biochem. Sci., *21:* 455–458, 1996.