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Expression of the *DMBT1* Gene Is Frequently Suppressed in Human Lung Cancer

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***DMBT1* (deleted in malignant brain tumors) is a candidate tumor suppressor gene that has been mapped to chromosome 10q25.3-q26.1, a region in which frequent loss of heterozygosity (LOH) has been observed in several human tumors. Since *DMBT1* is highly expressed in the lung, we analyzed LOH at the *DMBT1* locus and expression of this gene in lung cancer. Thirty-five (53%) of 66 primary lung cancers showed LOH, and diminished expression of *DMBT1* was observed in 20 (91%) of 22 lung cancer cell lines: three (14%) of them showed loss of expression. We further determined the primary structure of *DMBT1* and analyzed genetic alterations in this gene using 23 lung cancer cell lines. Two (9%) of them had homozygous deletion within the gene, and two cell lines had genetic aberrations: one was a rearrangement involving exons 5 and 6, and the other was a missense mutation at codon 52. These results suggest that inactivation of the *DMBT1* gene plays an important role in human lung carcinogenesis.**

Key words: *DMBT1* — SRCR family — Chromosome 10q — Human lung cancer

Allelic loss is a hallmark of inactivation of tumor suppressor genes. In lung cancer, loss of chromosome arms 1p, 2q, 3p, 5q, 7q, 9p, 10q, 13q, 16q, and 17p has been reported to date.[1–11] However, the responsible gene has not yet been identified in the great majority of these loci. *PTEN* was isolated from chromosome 10q23.3,[12–14] and frequent somatic mutation of this gene was observed in endometrial carcinomas[15–17] and endometrial hyperplasias,[18–20] the putative premalignant lesions of endometrial cancer. However, this gene was not frequently mutated in lung cancer in Japanese patients.[21] Recently, the *DMBT1* (deleted in malignant brain tumors) gene has been cloned from 10q25.3-q26.1.[22] DMBT1 encodes a protein that contains eight tandem repeats of the SRCR (scavenger receptor cysteine-rich) domains that are highly homologous, with 94–99% identity.[22] The SRCR protein group contains diverse members, some of which have been linked to the triggering of proliferation and/or differentiation processes[23–26]; one member, hensin, plays a role in switching the polarity of epithelial cells by mediating contacts between the extracellular matrix and cell-surface proteins.[27] The ZP domain is included in transforming growth factor β receptor type III (TGFβRIII), which regulates the association of TGF-β with the signaling receptors.[28, 29] Hence, there is a possibility that this gene works as a tumor suppressor.

Since the expression of the *DMBT1* gene was high in the lung, we hypothesized that genetic alteration of this gene is involved in human lung carcinogenesis. First we analyzed loss of heterozygosity (LOH) at the *DMBT1* locus in 66 primary lung cancer tissues (34 squamous cell carcinomas, 20 adenocarcinomas, 7 large cell carcinomas, and 5 small cell carcinomas) using a microsatellite marker D10S587. Tumors and corresponding normal tissues were frozen in liquid nitrogen immediately after resection and stored at –80°C until use. In each case, a part of the tumor specimen was fixed in formalin and examined histopathologically, and only specimens of lung cancers in which contamination by normal cells was less than 50% of the total were used for this analysis. Typical examples are shown in Fig. 1, and the results are summarized in Table I. Thirty-five (53%) of 66 lung cancers showed LOHs, and there was no association between incidence of LOH and histologic diagnosis.

We then characterized the genomic structure of *DMBT1*. Six overlapping BAC (bacterial artificial chromosome) clones covered the entire *DMBT1* gene (see Fig. 2). These BAC clones were purchased from Research Genetics (Huntsville, AL). The exon-intron boundaries were determined and it was found that this gene consists of 40 exons (see Fig. 2). The repeating regions encoding SRCR and

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SID (SRCR interspersed domain) were highly homologous not only in the exonic, but also in the intronic sequences. Each repeat unit consisting of one SRCR and one SID was encoded by three exons. Two candidate regions for exons that may encode additional SRCR domains were also found in intron 12 (see open boxes in Fig. 2). Both of the candidate exons are 324 bp in length with a continuous reading frame and flanked by AG and GT, being very similar to other SRCR-coding exons. These regions may be involved in alternative splicing mechanisms and may explain the previous finding of several different-sized *DMBT1* transcripts.[22] 5′-RACE using the lung mRNA was also performed and afforded a 464-base sequence upstream from the 5′ end of the published cDNA sequence: this sequence was identical with the genomic sequence. Nucleotide sequences of the exons, surrounding intronic regions, and 5′-flanking region of the gene have been deposited in the DDBJ, GenBank, and EMBL databases under the accession numbers AB020812 through AB020851.

We next analyzed the expression of *DMBT1* by reverse-transcription polymerase chain reaction (RT-PCR) in 22 human lung cancer cell lines.[30] A primer pair DM11 (5′-TCCAGGTCGAGGAAGTCCA-3′) in exon 39 and DM8

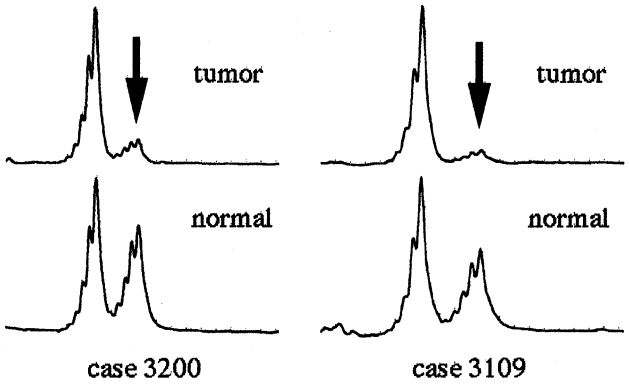

Fig. 1. PCR-LOH analysis at the *D10S587* locus in primary cancers of the lung. Typical examples of LOH are shown. Downward arrows indicate lost alleles in tumors.

Table I. Results of Microsatellite Analysis

	Patients	Informative cases	LOH (%)
Squamous cell carcinoma	46	34	17 (50)
Adenocarcinoma	28	20	13 (65)
Large cell carcinoma	8	7	2 (29)
Small cell carcinoma	8	5	3 (60)
Total	90	66	35 (53)

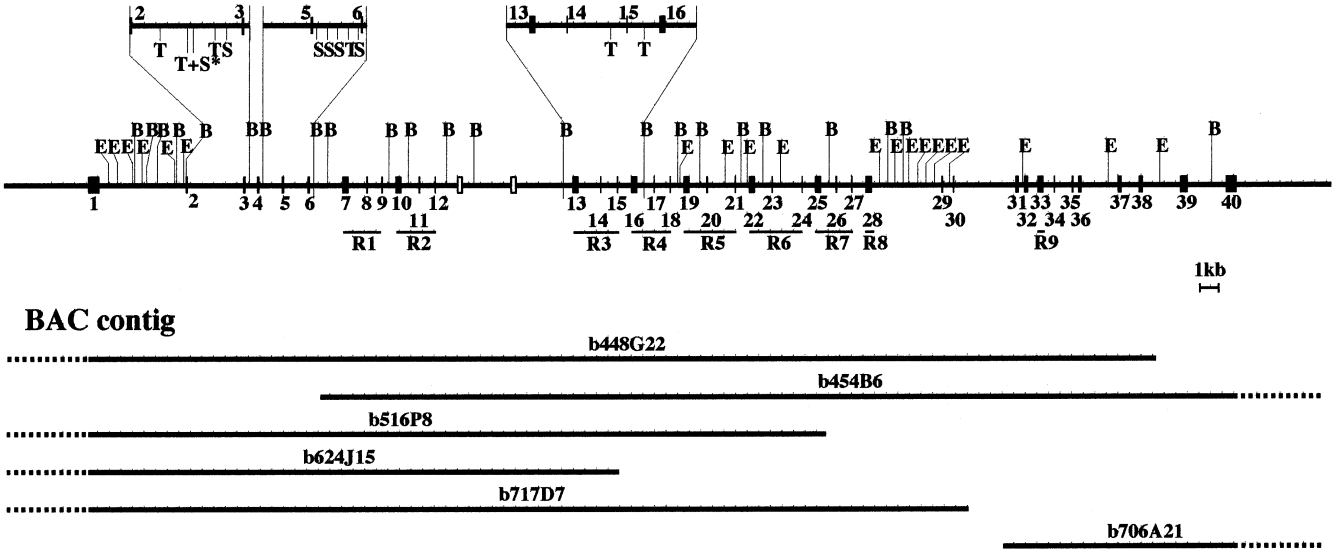

Fig. 2. Primary structure of the *DMBT1* gene. Locations of exons as well as introns are shown. Closed boxes indicate exons, and open boxes indicate two candidates for extra exons encoding SRCR domains found in the genomic sequence that were not included in the cDNA sequence already reported. Restriction endonuclease recognition sites that are relevant are also shown: B, *Bam*HI; E, *Eco*RI; S, *Sau*3AI; T, *Taq*I. Each repeat consisting of SRCR and SID is composed of three exons, and exons 7 through 28 represent eight stretches of the tandem repeat. An asterisk indicates one *Taq*I site and one *Sau*3AI site whose locations were not precisely determined. A contig consisting of six overlapping genomic BAC clones is also shown. A size standard is indicated below the line.

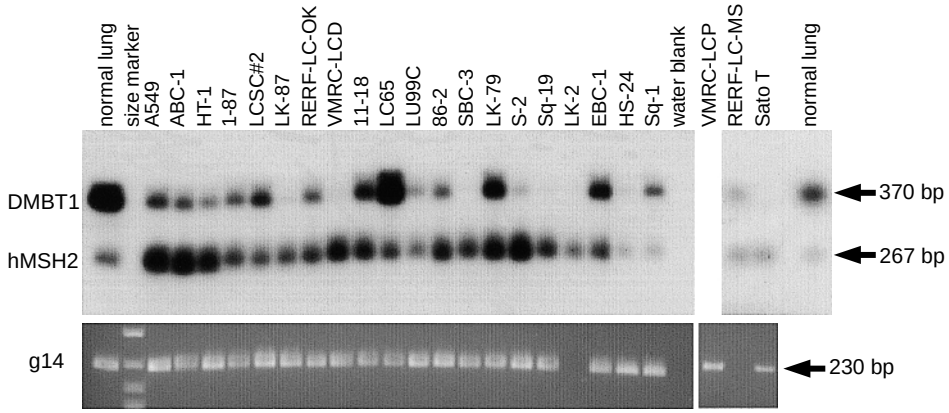

Fig. 3. (A) RT-PCR analysis of the *DMBT1* gene in human lung cancer cell lines. The majority of the cell lines showed diminished expression: among these, SBC-3, LK-2 and Sato T showed loss of expression. Expression of *hMSH2* was monitored as the control, and primers used were MC23 (5′-TGACTTCTCCAAGTTTCAGGA-3′) and MG2 (5′-CGAAGGACTTTTTCTTCCTTAC-3′) in exons 8 and 10, respectively. (B) Homozygous deletions were observed in two lung cancer cell lines by PCR amplification using primer pair g14 in intron 12. No PCR products were observed in LK-2 and RERF-LC-MS.

Table II. Primer Sequence for Exon Amplification and PCR Conditions

Exon	Forward primer	Nucleotide sequence (5′→3′)	Reverse primer	Nucleotide sequence (5′→3′)	Size of product (bp)	Annealing temp. (°C)	DMSO[a] (10%)
1	DM15	CAATCAATCAAACACACCTAAG	IDM2	AAAGTAGTAGATATAATTTGCCAT	242	56	(+)
2	IDM77	GCTTTAATCCGCTATTGCAG	IDM16	AGCTGTGCTCAGCCTCAAC	291	56	(+)
3	IDM79	GGCCATTTCAGTGATGGAGC	IDM22	AATGCCAGAGTTGATAGAAG	324	54	(+)
4	IDM9	GGGGCCAGTACGGTGACAC	IDM24	CACTACCTGAGAACATGACA	341	56	(+)
5	IDM15	GATGCTTGTGCGTCACCTTG	IDM26	CACTACCTGAGCACCTGACG	369	58	(+)
6	IDM17	GGCCAGTACAATGACAAAGC	IDM46	TCAGACCTGAGGCCTTTTGTG	339	60	(+)
7	IDM13	GCAGGCCCTGAGACTTTGTG	IDM84	TCATCCTCCCAAAGTGTGGG	460	58	(+)
8	IDM19	GGCATCAGCTCAGGGTGTAG	IDM30	GAGTATCTGCAGAGAAGCAG	229	58	(+)
9	IDM25	GCAAGTGGCCAGACCTTCG	IDM32	GTGAACTATGAAATCCAAAGTG	238	54	(+)
10, 16, 25, 28	IDM27[b]	TACC(C/T)TGAGTGTGGAAC	IDM34	CTTTGAGTGAGGAGATCAG	497	52	(+)
11, 17, 20, 23	IDM29	CATTTCTTTCCCTCCTC	IDM36	CAGTATCCACACAGAGAAACA	210	54	(+)
12, 18, 21, 24	IDM31	TGGATGAGTTCACAGCA	IDM38	CATCCTATCAGTTTCAAACAC	274	52	(+)
13	IDM33	CTTTTCCTTCACCTCAGAATG	IDM40	CAGAACATAATGAGACCTCG	455	54	(+)
14	IDM35	TGAAGGCATGATCTGTTTAG	IDM48	TGCAGAAAGGGATAATTATAC	186	56	(–)
15	IDM37	CCTTCTCCGGAGACCTTTCC	IDM44	CCTATCATTTTCAGAAAAAACA	191	52	(+)
19, 22	IDM45	ACTTGACTACTTTGCCCAC	IDM58	TAGAAGAATCTGTGAGTTCAG	524	54	(+)
26	IDM59	GGCTTGCCCAGTTCCTTCTAT	IDM66	TTACAGAGTGGTGGGAGGAA	254	58	(+)
27	IDM61	TTTTTTGTAGCTTTCCTCCC	IDM68	TCCCCAGGCAACTCGCGGAA	228	54	(+)
29	IDM65	CTTTCAGGCTGTAAGAATG	IDM72	AGTGATGGCAGTGGGAACTC	390	54	(+)
30	IDM23	CTGAGAAGTCCTGTACTAAGG	IDM74	CCCTTCTTCCTCCTAAAGC	382	56	(+)
31	IDM91	TAAAGTGCAGAAGATGAAACTGG	IDM4	AAACCAGTGGGTGTGCTGC	318	62	(+)
32	IDM3	TATAAAATGGAGGGGCAATAGG	IDM12	TGCCCCCAAAGAGGCAGC	328	56	(+)
33-5′	IDM81	GGTTTGTTGTGGGACATTTG	DM48	TGGGAGAACCAGCCTCGG	343	60	(+)
33-3′	DM39	TTCAGCCCTTGGAAATGCATA	IDM76	AGCCACTGGCCTTAAGCTCTTG	234	60	(+)
34	IDM69	TGACGGGACTTAGGGAGCATC	IDM78	TGAACTCCTTGGAGAGTGAG	309	58	(+)
35	IDM21	GCTTTGGTCAATCTCATCC	IDM18	TGGCAGGGCACTCTTGCAG	375	56	(+)
36	IDM93	TCCAGATCTAGGCCACCTCTTG	IDM10	AGCACCATTTATTCCCTCTTG	369	60	(+)
37	IDM5	ATCTGACCCCCTGCGTCAA	IDM92	ACAGATCCTCTCAGGATGTCC	331	60	(+)
38-5′	IDM73	GAAATCCTGAGTCCACGTGC	DM52	AATATCACCAGGTTCGGCGT	377	60	(+)
38-3′	DM77	CTGTCTGCCAAATCACATGC	IDM82	CCTTTATTCCAATTATGTCCTG	309	56	(+)
39-5′	IDM75	TAGCAGGTGACATGTGCCTG	DM6	GCCTGAGTGTTGACTGAGG	317	60	(+)
39-3′	DM11	TCCAGGTCGAGGAAGTCCA	IDM50	TACTGGGAACTGCAGTTCATC	347	60	(+)
40-5′	IDM7	GTACAACTGAGTCATGAAGG	DM8	CTGCACACCACCATTTTACA	199	58	(+)
40-3′	DM13	TCGCCGTCTCTTCGCATTG	DM10	CCCAAGTCCCCGAGTCAG	303	60	(+)

a) DMSO, dimethyl sulfoxide.
b) IDM27 is a mixture of 5′-TACCCTGAGTGTGGAAC-3′ and 5′-TACCTTGAGTGTGGAAC-3′.

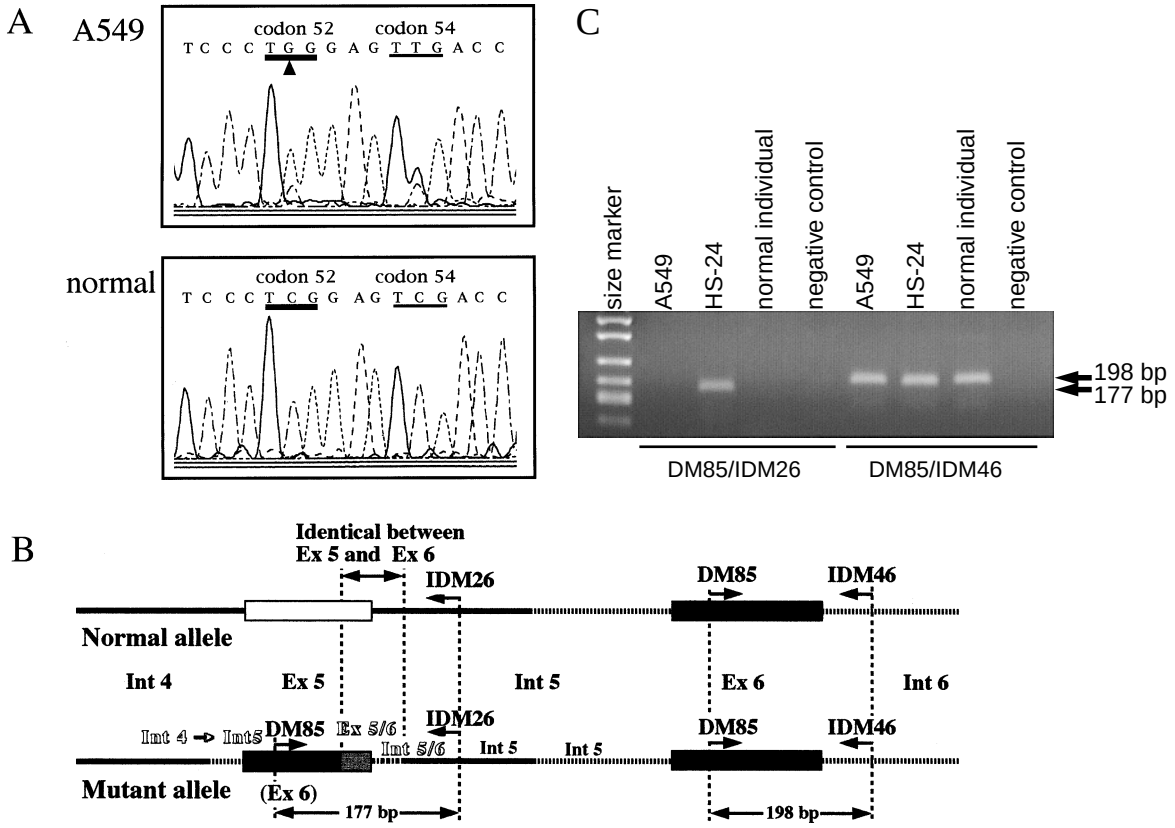

Fig. 4. (A) Results of nucleotide sequencing analysis around codon 52 in DNA samples of a normal volunteer and A549. Base substitutions are indicated by thick (codon 52) and thin (codon 54) underlines. The nucleotide change at codon 52 causes a missense mutation from Ser to Trp (from TCG to TGG as indicated by an arrowhead) and that at codon 54 is a Ser/Leu polymorphism (TCG/TTG). These alterations were analyzed by Wu *et al*.[31] (B) A rearrangement found in HS-24 involving exons 5 and 6 is schematically illustrated. A portion harboring exon 5 was replaced by that of exon 6 in one of the alleles in this cell line. The breakpoint at the 3′ part of the rearrangement is ambiguous due to the identity of sequences between exons 5 and 6, as well as introns 5 and 6. This rearrangement would cause amino acid changes from Pro to Leu at codon 65, from Ser to Pro at codon 67, and from Leu to Ser at codon 68. (C) The rearrangement in HS-24 was confirmed by PCR amplification. Primers used are indicated in the schema in (B).

(5′-CTGCACACCACCATTTACA-3′) in exon 40 was used. Twenty (91%) of 22 cell lines showed diminished expression: among these, three (SBC-3, LK-2 and Sato T) showed loss of expression (see Fig. 3A). Expression of *hMSH2* was monitored as the control.

Homozygous deletions were also surveyed by PCR amplification of exons 1, 29, 35, 39, and intron 12 using the 22 cell lines as well as one additional lung cancer cell line (VMRC-LCP). As shown in Fig. 3B, two cell lines, LK-2 and RERF-LC-MS, did not produce any PCR products when we used two primer pairs g14 (primers g14f2 and g14r3) and g14ext (primers g14extf2 and g14extr1), both in intron 12.[22] These results strongly suggested homozygous deletion, but the possibility of insertion/deletion polymorphism could not be totally excluded. We could not identify the precise region of the homozygous deletion due to the very high homology of the genomic sequence surrounding intron 12. LK-2, one of the cell lines with homozygous deletion, also showed loss of expression of *DMBT1*.

We further examined genetic alterations of the *DMBT1* gene in 23 lung cancer cell lines. Primers used for mutation analyses are shown in Table II. Due to the high homology in exonic as well as intronic sequences in the repeated region, it was not possible to amplify some exons individually. The entire coding exons as well as surrounding regions of the gene were surveyed, and two mutations were found as shown in Fig. 4. In A549, a heterozygous missense mutation from TCG to TGG at codon 52 that causes an amino acid change from Ser to Trp was observed (see Fig. 4A). This mutation was also reported in a lung cancer cell line Calu-1 by Wu *et al*.[31] HS-24 harbored a rearrangement involving exons 5 and 6 in one of the alleles as schematically shown in Fig. 4B. Although

exons 5 and 6 are highly homologous, some amino acid alterations may influence the function of the DMBT1 protein. This rearrangement, confirmed by PCR amplification as shown in Fig. 4C, was not observed in DNA samples of 50 normal volunteers.

In summary, the great majority of lung cancer cell lines showed diminished expression or loss of expression of *DMBT1*, and two (9%) of 23 cell lines had a homozygous deletion. Genetic alteration was also observed in two cell lines. While we were preparing the manuscript, Wu *et al*. reported frequent loss of expression of *DMBT1* in lung cancer.[31] Our results along with those of Wu *et al*. suggested that inactivation of the *DMBT1* gene plays an important role in lung carcinogenesis.

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REFERENCES

1) Petersen, I., Bujard, M., Petersen, S., Wolf, G., Goeze, A., Schwendel, A., Langreck, H., Gellert, K., Reichel, M., Just, K., du Manoir, S., Cremer, T., Dietel, M. and Ried, T. Patterns of chromosomal imbalances in adenocarcinoma and squamous cell carcinoma of the lung. *Cancer Res.*, **57**, 2331–2335 (1997).
2) Balsara, B. R., Sonoda, G., du Manoir, S., Siegfried, J. M., Gabrielson, E. and Testa, J. R. Comparative genomic hybridization analysis detects frequent, often high-level, overrepresentation of DNA sequences at 3q, 5p, 7p, and 8q in human non-small cell lung carcinomas. *Cancer Res.*, **57**, 2116–2120 (1997).
3) Todd, S., Franklin, W. A., Varella-Garcia, M., Kennedy, T., Hilliker, C. E., Jr., Hahner, L., Anderson, M., Wiest, J. S., Drabkin, H. A. and Gemmill, R. M. Homozygous deletions of human chromosome 3p in lung tumors. *Cancer Res.*, **57**, 1344–1352 (1997).
4) Wiest, J. S., Franklin, W. A., Otstot, J. T., Forbey, K., Varella-Garcia, M., Rao, K., Drabkin, H., Gemmill, R., Ahrent, S., Sidransky, D., Saccomanno, G., Fountain, J. W. and Anderson, M. W. Identification of a novel region of homozygous deletion on chromosome 9p in squamous cell carcinoma of the lung: the location of a putative tumor suppressor gene. *Cancer Res.*, **57**, 1–6 (1997).
5) Fong, K. M., Zimmerman, P. V. and Smith, P. J. Tumor progression and loss of heterozygosity at 5q and 18q in non-small cell lung cancer. *Cancer Res.*, **55**, 220–223 (1995).
6) Shiseki, M., Kohno, T., Nishikawa, R., Sameshima, Y., Mizoguchi, H. and Yokota, J. Frequent allelic losses on chromosome 2q, 18q, and 22q in advanced non-small cell carcinoma. *Cancer Res.*, **54**, 5643–5648 (1994).
7) Merlo, A., Gabrielson, E., Mabry, M., Vollmer, R., Baylin, S. B. and Sidransky, D. Homozygous deletion on chromosome 9p and loss of heterozygosity on 9q, 6p, and 6q in primary human small cell lung cancer. *Cancer Res.*, **54**, 2322–2326 (1994).
8) Ried, T., Petersen, I., Holtgreve-Grez, H., Speicher, M. R., Schrock, E., du Manoir, S. and Cremer, T. Mapping of multiple DNA gains and losses in primary small cell lung carcinomas by comparative genomic hybridization. *Cancer Res.*, **54**, 1801–1806 (1994).
9) Sato, M., Mori, Y., Sakurada, A., Fukushige, S., Ishikawa, Y., Tsuchiya, E., Saito, Y., Nukiwa, T., Fujimura, S. and Horii, A. Identification of a 910 kb region of common allelic loss in chromosome bands 16q24.1-q24.2 in human lung cancer. *Genes Chromosom. Cancer*, **22**, 1–8 (1998).
10) 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).
11) Petersen, S., Wolf, G., Bockmuhl, U., Gellert, K., Dietel, M. and Petersen, I. Allelic loss on chromosome 10q in human lung cancer: association with tumour progression and metastatic phenotype. *Br. J. Cancer*, **77**, 270–276 (1998).
12) 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 beta. *Cancer Res.*, **57**, 2124–2129 (1997).
13) 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).
14) 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).
15) Kong, D., Suzuki, A., Zou, T.-T., Sakurada, A., Kemp, L. W., Wakatsuki, S., Yokoyama, T., Yamakawa, H., Furukawa, T., Sato, M., Ohuchi, N., Sato, S., Yin, J., Wang, S., Abraham, J. M., Souza, R. F., Smolinski, K. N., Meltzer, S. J. and Horii, A. *PTEN1* is frequently mutated in primary endometrial carcinomas. *Nat. Genet.*, **17**, 143–144 (1997).
16) Risinger, J. I., Hayes, A. K., Berchuck, A. and Barrett, J. C. *PTEN/MMAC1* mutations in endometrial cancers. *Cancer Res.*, **57**, 4736–4738 (1997).
17) Tashiro, H., Blazes, M. S., Wu, R., Cho, K. R., Bose, S.,

Wang, S. I., Li, J., Parsons, R. and Ellenson, L. H. Mutations in *PTEN* are frequent in endometrial carcinoma but rare in other common gynecological malignancies. *Cancer Res.*, **57**, 3935–3940 (1997).
18) Levine, R. L., Cargile, C. B., Blazes, M. S., van Rees, B., Kurman, R. J. and Ellenson, L. H. *PTEN* mutations and microsatellite instability in complex atypical hyperplasia, a precursor lesion to uterine endometrioid carcinoma. *Cancer Res.*, **58**, 3254–3258 (1998).
19) Maxwell, G. L., Risinger, J. I., Gumbs, C., Shaw, H., Bentley, R. C., Barrett, J. C., Berchuck, A. and Futreal, P. A. Mutation of the *PTEN* tumor suppressor gene in endometrial hyperplasias. *Cancer Res.*, **58**, 2500–2503 (1998).
20) Yoshinaga, K., Sasano, H., Furukawa, T., Yamakawa, H., Yuki, M., Sato, S., Yajima, A. and Horii, A. The *PTEN*, *BAX*, and *IGFIIR* genes are mutated in endometrial atypical hyperplasia. *Jpn. J. Cancer Res.*, **89**, 985–990 (1998).
21) Sakurada, A., Suzuki, A., Sato, M., Yamakawa, H., Orikasa, K., Uyeno, S., Ono, T., Ohuchi, N., Fujimura, S. and Horii, A. Infrequent genetic alterations of the *PTEN/MMAC1* gene in Japanese patients with primary cancers of the breast, lung, pancreas, kidney, and ovary. *Jpn. J. Cancer Res.*, **88**, 1025–1028 (1997).
22) Mollenhauer, J., Wiemann, S., Scheurlen, W., Korn, B., Hayashi, Y., Wilgenbus, K. K., von Deimling, A. and Poustka, A. *DMBT1*, a new member of the SRCR superfamily, on chromosome 10q25.3-26.1 is deleted in malignant brain tumours. *Nat. Genet.*, **17**, 32–39 (1997).
23) Law, S. K. A., Micklem, K. J., Shaw, J. M., Zhang, X. P., Dong, Y., Willis, A. C. and Mason, D. Y. A new macrophage differentiation antigen which is a member of the scavenger receptor superfamily. *Eur. J. Immunol.*, **23**, 2320–2325 (1993).
24) Ullrich, A., Sures, I., D'Egidio, M., Jallal, B., Powell, T. J., Herbst, R., Dreps, A., Azam, M., Rubinstein, M., Natoli, C., Shawver, L. K., Schlessinger, J. and Iacobelli, S. The secreted tumor-associated antigen 90K is a potent immune stimulator. *J. Biol. Chem.*, **269**, 18401–18407 (1994).
25) Whitney, G. S., Starling, G. C., Bowen, M. A., Modrell, B., Siadak, A. W. and Aruffo, A. The membrane-proximal scavenger receptor cysteine-rich domain of CD6 contains the activated leukocyte cell adhesion molecule binding site. *J. Biol. Chem.*, **270**, 18187–18190 (1995).
26) Starling, G. C., Whitney, G. S., Siadak, A. W., Llewellyn, M. B., Bowen, M. A., Farr, A. G. and Aruffo, A. A. Characterization of mouse CD6 with novel monoclonal antibodies which enhance the allogeneic mixed leukocyte reaction. *Eur. J. Immunol.*, **26**, 738–746 (1996).
27) Al-Awqati, Q. Plasticity in epithelial polarity of renal intercalated cells: targeting of the H(+)-ATPase and band 3. *Am. J. Physiol.*, **270**, C1571–1580 (1996).
28) Bork, P. and Sander, C. A large domain common to sperm receptors (Zp2 and Zp3) and TGF-beta type III receptor. *FEBS Lett.*, **300**, 237–240 (1992).
29) Sun, L. and Chen, C. Expression of transforming growth factor beta type III receptor suppresses tumorigenicity of human breast cancer MDA-MB-231 cells. *J. Biol. Chem.*, **272**, 25367–25372 (1997).
30) Mori, Y., Shiwaku, H., Fukushige, S., Wakatsuki, S., Sato, M., Nukiwa, T. and Horii, A. Alternative splicing of *hMSH2* in normal human tissues. *Hum. Genet.*, **99**, 590–595 (1997).
31) Wu, W., Kemp, B. L., Proctor, M. L., Gazdar, A. F., Minna, J. D., Hong, W. K. and Mao, L. Expression of *DMBT1*, a candidate tumor suppressor gene, is frequently lost in lung cancer. *Cancer Res.*, **59**, 1846–1851 (1999).