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Mutational Analysis of the *Macrophage Scavenger Receptor 1* (*MSR1*) Gene in Primary Lung Cancer

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Abstract

Allelic deletion at chromosome 8p21-25 is an early and frequent event in the carcinogenesis and development of various cancers. To facilitate investigation of alterations of the *macrophage scavenger receptor 1* (*MSR1*), which is located on 8p22, and to determine the role of this gene in human carcinogenesis and tumor progression, we determined intronic primers designed to amplify the coding region. Since frequent deletion of 8p21-23 has been previously reported in lung cancer, we searched for mutations throughout the coding sequence of the *MSR1* gene within a panel of genomic DNA samples obtained from 30 primary lung cancers. Our approach, which involved polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis and direct DNA sequencing, revealed nucleotide variants of the *MSR1* gene in only one of the 30 cases examined, with this sample displaying both a 6 bp deletion and a thymine-to-cytosine substitution, the latter occurring within intron 7. The 6 bp deletion was located at a DNA microsatellite region and the thymine-to-cytosine substitution seemed to be a polymorphism.

These results suggest that the *MSR1* gene is not commonly mutated in lung cancer and not important in susceptibility to lung cancer. Further studies may focus on alternative mechanisms through which the *MSR1* gene might be inactivated, such as aberrant DNA methylation, and/or pursue analyses of other genes on 8p21-23 for mutational events. Nevertheless, the panel of intronic PCR primer pair sequences presented here will facilitate future studies to determine the full spectrum and frequency of genetic events that may affect expression/activity of the *MSR1* gene in human tumors.

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Key words: lung cancer, deletion, *macrophage scavenger receptor 1* (*MSR1*) gene, mutation, polymorphism

Introduction

The development of human cancers including lung

cancers is associated with an accumulation of genetic alterations of tumor suppressor genes. Cytogenetic and molecular studies of cancer tissue specimens have revealed chromosomal deletions in

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various regions^{1,2}. Frequent loss of heterozygosity (LOH) at a specific chromosomal region in certain tumors, as reported in many studies, implies the presence of tumor suppressor genes (TSGs) associated with their tumorigenesis. Allelic losses of 3p, 5q, 8p, 9p, 11p, 13q, and 17p have been reported in primary lung cancer³⁻¹². In agreement, the *p16* gene (chromosome 9p)¹³⁻¹⁵, the *Rb* gene (chromosome 13q)^{16,17}, and the *p53* gene (chromosome 17p)¹⁸⁻²⁰ are all reported to be inactivated in lung cancer. The involvement of *FHIT* on chromosome 3p as a TSG in lung cancer has also been proposed¹⁰⁻¹². Recently, genetic alterations on the short arm of chromosome 8 (either LOH or chromosomal deletions) representing a frequent event in several cancers including prostate^{21,22}, breast^{23,24}, colon²⁵, lung^{26,27}, head and neck²⁸ cancer and hepatocellular carcinoma²⁵, have been documented. In our previous study, which involved LOH analysis of chromosome 8p using ten microsatellite markers, the overall allelic deletion rates of 8p21-23 were 10 of 28 (35.7%)²⁹. The allelic deletions of this locus in the primary cancer and its metastatic sites were in each case identical in both frequency and size of the deleted regions. Our previous results, therefore, suggest that allelic deletion at chromosome 8p21-23 represents an early event frequently occurring in the development of lung cancer, but rarely contributes to its metastasis. Therefore, this region may harbor tumor suppressor genes of lung cancer.

Macrophage scavenger receptor 1 (MSR1), whose gene is located at 8p22, functions in several processes proposed to be relevant to prostate carcinogenesis. The germline mutations and sequence variants of the *MSR1* gene were also reported to be important in susceptibility to prostate cancer³⁰. The *MSR1* gene seems to be a potential candidate as a TSG in lung cancer and alteration of the gene may be involved in the genesis and progression of primary lung cancer. To test this hypothesis, we screened for mutations within the *MSR1* gene using polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis, and direct DNA sequencing, of tissues obtained from patients with primary lung cancer.

Materials and Methods

Tissue samples

This study included 30 subjects with primary lung cancer. The patients were seen at the Fourth Department of Internal Medicine at Nippon Medical School Main Hospital in Tokyo, Japan²⁰. From each subject, we obtained tissue samples from the primary lung cancer site, any distant metastatic site, and normal lung tissue at the time of autopsy from 1989 to 1995. Informed consents for research use from the patients or their family were obtained by the Fourth Department of Internal Medicine. The metastatic site from which we obtained samples in the 30 cases included lung (13 cases), liver (10 cases), pleura (3 cases), subcutaneous or muscle tissue (2 cases), spleen (1 case), and pancreas (1 case). The histologic type of lung cancers included 6 small cell carcinomas, 14 adenocarcinomas, 8 squamous cell carcinomas, 1 large cell carcinoma and 1 adenosquamous cell carcinoma. Genomic DNA was obtained from each tissue sample by proteinase K treatment and phenol-chloroform extraction according to the standard protocols^{15,20}.

PCR-SSCP analysis

Mutations in exons of the *MSR1* gene were examined by PCR-SSCP analysis. From each genomic DNA sample, exons of the *MSR1* gene were amplified separately using the PCR primer pairs listed in **Table 1**. Each PCR reaction contained 25 pmol of exon-specific primer pairs labeled with fluorescein isothiocyanate (FITC), 2.5 mM dNTPs, 1.25 units of Ex Taq DNA polymerase, and 1x Ex Taq buffer (Takara, Tokyo, Japan) in a final reaction volume of 20 μ l. The 20- μ l reaction mixture was placed in a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) when the heating block reached 94°C. Standard denaturing and extension temperatures of 94°C and 72°C, respectively, were used. The annealing temperature was 57°C with the PCR primers designed in this study, and the number of cycles was 35 (exon 2, 3, 5~9) and 45 (exon 4 (No. 1, 2)), respectively. FITC-labeled PCR products were denatured, cooled on ice, and loaded on neutral

Table 1 List of the intron-based primer sequences used in the PCR amplification of each exon of *MSRI* gene coding region

	Sense primer sequence	Antisense primer sequence
Exon 2	TATGCAAAAGAGCCCTAGC	GTCAATAACTCTAAGAACAACCTCC
Exon 3	CACTTTCTAACAGTAGGCTGGGC	TGCTTCTTTTTTGCTTTGGC
Exon 4 (No.1)	GGAATGATATGGGATGGGATC	AGGACTTGAGATTTTCATC
Exon 4 (No.2)	TTGTTTTCTCAGTCCAGGG	ATTCAGTTCAGCAAGTGACC
Exon 5	CAAAGGCTGTGAATGCTC	TCTGGAGAAAATGACAAGAC
Exon 6	ACCTTGACAGATGACTAACCC	TAGCAATCCTCCCCTACAC
Exon 7	CTCTCAAGTCTGTATGAAAATG	GGTGAACAGAAGTCAGTTTC
Exon 8	CCTTGTGATGATCTGTCTCC	ATTTGCCTCAAGCCCAG
Exon 9	CCCCACATCTGTAGTTAATGG	GTCATTTGGAGGAGTCAC

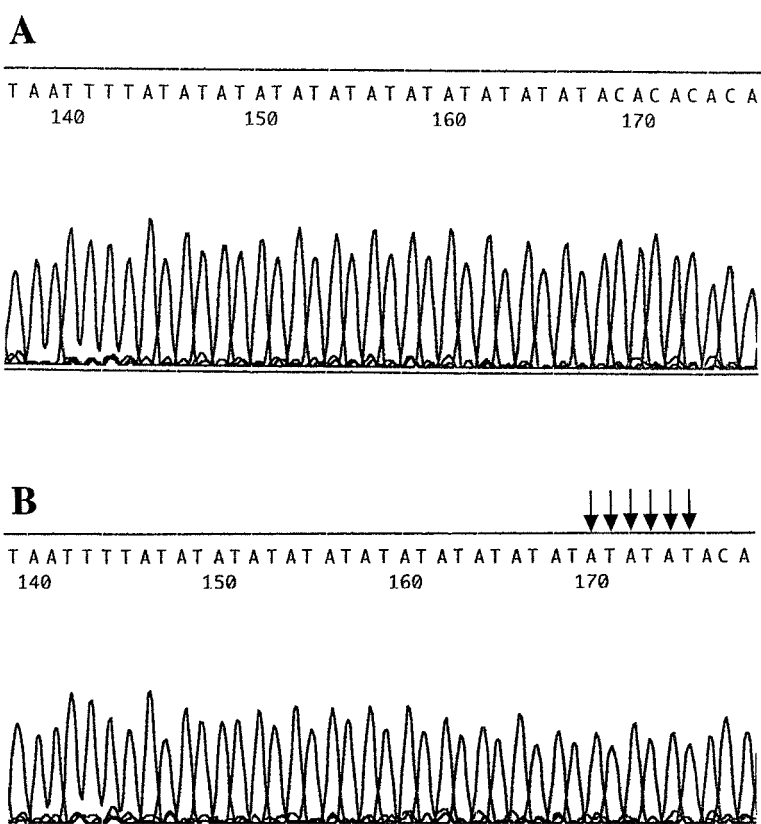


Fig. 1 DNA sequence analysis of the aberrant bands identified by PCR-SSCP analysis for exon 7 within the *MSRI* gene in case 21 (A), normal band of case 21 (B). These results reveal 6 bp deletion in an allele of case 21. The arrows indicate the site of deletion.

6% polyacrylamide gels with or without 5% (vol/vol) glycerol, as described previously^{15,20}. After electrophoresis, the gels were analyzed with the FluorImager (Amersham Pharmacia Biotech, Uppsala, Sweden). Aberrant bands were excised and further amplified by PCR for DNA sequence

analysis.

DNA sequence analysis

Each DNA sample was amplified by PCR using all primer pairs, with the-21 m13 forward primer sequence (TGTAACACGACGGCCAGT) added in

each case to the appropriate primer. PCR amplification was performed as described above; the products were purified and sequenced by fluorescent automated sequencing and DNA sequencing kit (Big Dye™ Cycle Sequencing Primer Ready Reaction-21M13) (Perkin Elmer/Applied Biosystems, Foster City, CA).

Results

Design of primer pairs for mutational analysis of the *MSR1* gene

We determined the nucleotide sequences of exons 1 through 11 of the *MSR1* gene, along with adjacent introns, by comparing the genomic sequence of *MSR1* with its respective cDNA sequence (GenBank accession no. NM 138716). All of the exon-intron boundaries within the *MSR1* gene conformed to the "AT-GT rule". The open reading frame (ORF) spanned from exon 2 to 9. Accordingly, we designed nine primer pairs based on intronic sequences flanking each exon of the *MSR1* gene, so as to permit analysis of the entire coding region (Table 1). Exon 4 was divided into two regions for analysis. Antisense primer for exon 4 No. 1 and sense primer for exon 4 No. 2 were exonic primers.

Mutational analysis of the *MSR1* gene in primary lung cancers

Tumor specimens from primary lung cancer patients were examined for mutations within the *MSR1* gene by PCR-SSCP analysis and DNA sequencing. The DNA sequence of *MSR1* in the matched tumor and normal tissue samples from each patient showed the same pattern on PCR-SSCP. In case 21, PCR-SSCP analysis of exon 7, together with intron sequence flanking this exon, revealed an aberrant banding pattern, with normal bands being evident. DNA sequence analysis of these aberrant bands showed a 6 bp deletion within a dinucleotide (AT) repeat in the tumor tissue as compared with other patients (Fig. 1). Although this poly (AT) tract has 15 AT repeats in the non-coding region, 60 bp downstream of the coding ending point of exon 7, in the main population the normal and tumor tissues of case 21 had 12 and 15 AT repeats (Fig. 1). A

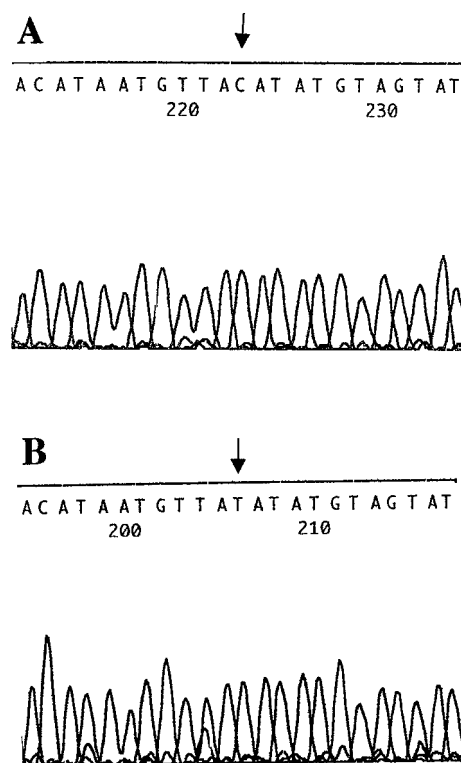


Fig. 2 DNA sequence data reveal an T-to-C nucleotide substitution within intron 7 in case 21. The arrows indicate the site of substitution. Aberrant DNA sequence (A); control DNA sequence (B).

thymine-to-cytosine substitution of intron 7, 63 bp downstream of the coding ending point of exon 7, was also revealed by DNA sequencing in case 21 (Fig. 2). The diagnosis of case 21 was adenocarcinoma. Overall, the frequency of variants within this panel of lung tumors was 3.3%. These tumor tissues were not amenable for mRNA expression analysis. No other abnormal bands were detected in the tumor specimens, as determined by PCR-SSCP analysis.

Discussion

In our previous study, we observed LOH on 8p21-23 in 10 out of 28 (35.7%) informative cases examined, which is as frequent as for 3p14.2 and 3p21-25 (11 of 28 (39.3%))²⁹. Allelic deletion at chromosome 8p21-23, independent of chromosome 3p deletion, was also an early and frequent event in the development of lung cancer. However, the TSGs

that might reside on 8p21-25 are as yet unknown. Recently, germline mutations and sequence variants of the *MSR1* gene were reported to be associated with prostate cancer, which was reported to be deleted on chromosome 8p22-23³⁰. As such, *MSR1* seems to be a potential candidate as a TSG in lung cancer. In order to evaluate the role of the *MSR1* gene in lung cancer development, we carried out mutation analysis of the gene in a panel of lung cancers. However, the results of the present study indicated that mutation of the *MSR1* gene might not be common in lung cancers. We detected nucleotide variants of the *MSR1* gene in only one of 30 cases, with one of these variations being an thymine-to-cytosine substitution in intron 7 that would not influence the transcriptional sequence of *MSR1* in the tumor. The other nucleotide variation, present in the same case, was a 6 bp deletion in a microsatellite repeat sequence. Interestingly, this tumor also displayed microsatellite instability (data not shown). On the basis of previously published results, the frequency of microsatellite instability in lung cancer varies considerably, ranging from 2 to 30%^{31,32}. The normal tissue from this case also had (AT)¹² and (AT)¹⁵ repeat at this sequence. The thymine-to-cytosine substitution and the presence of (AT)¹² repeat sequence were on other alleles.

With respect to lung cancer in general, mutation of the *MSR1* gene may not be associated with development of this tumor type. Therefore, future studies must also focus on other inactivated mechanisms of the gene, as well as on other candidate TSG genes residing on chromosome 8p21-25 which may be deleted/alterd in lung cancer. However, *MSR1*, through its induction by oxidative stress and its ability to bind oxidized low-density lipoprotein, may modify amounts of reactive oxygen intermediates. *MSR1* knockout mice that have a reduced capacity to eradicate certain pathogens may also be relevant, because an infectious etiology of some kind of cancers has been proposed. The intronic primer sequences reported here should facilitate future efforts to define the role of the *MSR1* gene in human carcinogenesis and tumor progression.

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References

1. Shiraishi M, Morinaga S, Noguchi M, Shimosato Y, Sekiya T: Loss of genes on short arm of chromosome 11 in human lung carcinomas. *Jpn J Cancer Res* 1987; 78: 1302-1308.
2. Petersen S, Wolf G, Bockmuhl U, Gellert K, Dietel M, Peterson I: Allelic loss on chromosome 10q in human lung cancer: association with tumor progression and metastatic phenotype. *Br J Cancer* 1998; 77: 270-276.
3. Sozzi G, Miozzo M, Tagliabue E, Calderone C, Lombardi L, Pilotti S, Pastorino U, Pierotti MA, Della Porta G: Cytogenetic abnormalities and overexpression of receptors for growth factors in normal bronchial epithelium and tumor samples of lung cancer patients. *Cancer Res* 1991; 51: 400-404.
4. Sundaresan V, Ganly P, Hasleton P, Rudd R, Sinha G, Bleehen NM, Rabbitts P: p53 and chromosome 3 abnormalities, characteristic of malignant lung tumours, are detectable in preinvasive lesions of the bronchus. *Oncogene* 1992; 7: 1989-1997.
5. Thiberville L, Payne P, Vielkinds J, LeRiche J, Horsman D, Nouvet G, Palcic B, Lam S: Evidence of cumulative gene losses with progression of premalignant epithelial lesions to carcinoma of the bronchus. *Cancer Res* 1995; 55: 5133-5139.
6. Hung J, Kishimoto Y, Sugio K, Virmani A, McIntire DD, Minna JD, Gazdar AF: Allele-specific chromosome 3p deletions occur at an early stage in the pathogenesis of lung carcinoma. *J Am Med Assoc* 1995; 273: 558-563.
7. Kishimoto Y, Sugio K, Hung JY, Virmani AK, McIntire DD, Minna JD, Gazdar AF: Allele-specific loss in chromosome 9p loci in preneoplastic lesions accompanying non-small-cell lung cancers. *J Natl Cancer Inst* 1995; 87: 1224-1229.
8. Sekido Y, Fong KM, Minna JD: Progress in understanding the molecular pathogenesis of human lung cancer. *Biochim Biophys Acta* 1998; 1378: F21-59.
9. Park IW, Wistuba II, Maitra A, Milchgrub S, Virmani AK, Minna JD, Gazdar AF: Multiple clonal abnormalities in the bronchial epithelium of patients with lung cancer. *J Natl Cancer Inst* 1999; 91: 1863-1868.
10. Sozzi G, Veronese ML, Negrini M, Baffa R, Cotticelli MG, Inoue H, Torielli S, Pilotti S, De Gregorio L, Pastorino U, Pierotti MA, Ohta M, Huebner K, Croce CM: The FHIT gene 3p14.2 is abnormal in lung cancer. *Cell* 1996; 85: 17-26.
11. Sozzi G, Pastorino U, Moiraghi L, Tagliabue E, Pezzella F, Ghirelli C, Torielli S, Sard L, Huebner K, Pierotti MA, Croce CM, Pilotti S: Loss of FHIT function in lung cancer and preinvasive bronchial lesions. *Cancer Res* 1998; 58: 5032-5037.

12. Burke L, Khan MA, Freedman AN, Gemma A, Rusin M, Guinee DG, Bennett WP, Caporaso NE, Fleming MV, Travis WD, Colby TV, Trastek V, Pairolero PC, Tazelaar HD, Midthun DE, Liotta LA, Harris CC: Allelic deletion analysis of the FHIT gene predicts poor survival in non-small cell lung cancer. *Cancer Res* 1998; 58: 2533-2536.
13. Okamoto A, Demetrick DJ, Spillare EA, Hagiwara K, Hussain SP, Bennett WP, Forrester K, Gerwin B, Serrano M, Beach DH, Harris CC: Mutations and altered expression of *p16^{CKD2}* in human cancer. *Proc Natl Acad Sci USA* 1994; 91: 11045-11049.
14. Nakagawa K, Conrad NK, Williams JP, Johnson BE, Kelley MJ: Mechanism of inactivation of *CDKN2* and *MTS2* in non-small cell lung cancer and association with advanced stage. *Oncogene* 1995; 11: 1843-1851.
15. Gemma A, Takenoshita S, Hagiwara K, Okamoto A, Spillare EA, MacMenamin MG, Hussain SP, Forrester K, Zariwala M, Xiong Y, Harris CC: Molecular analysis of the cyclin-dependent kinase inhibitor genes *p15^{INK4B/MTS2}*, *p16^{INK4/MTS1}*, *p18* and *p19* in human cancer cell lines. *Int J Cancer* 1996; 68: 605-611.
16. Xiong Y, Zhang H, Beach D: D type cyclins associate with multiple protein kinases and the DNA replication and repair factor PCNA. *Cell* 1992; 71: 505-514.
17. Serrano M, Honnon GJ, Beach D: A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature* 1994; 366: 704-707.
18. Hollstein M, Sidransky D, Vogelstein B, Harris CC: *p53* mutation in human cancers. *Science* 1991; 253: 49-53.
19. Takahashi T, Carbone D, Nau MM, Hida T, Linnoila I, Ueda R, Minna JD: Wild-type but not mutant *p53* suppresses the growth of human lung cancer cells bearing multiple genetic lesions. *Cancer Res* 1992; 52: 2340-2343.
20. Taniguchi Y, Gemma A, Takeda Y, Takenaka K, Niitani H, Kudoh S, Shimada T: Stability of *p53* tumor suppressor gene mutations during the process of metastasis and during chemotherapy. *Lung Cancer* 1996; 14: 219-228.
21. Kagan J, Stein J, Babaian RJ, Joe YS, Pisters LL, Glassman AB, von Eschenbach AC, Troncoso P: Homozygous deletions at 8p22 and 8p21 in prostate cancer implicate these regions as the sites for candidate tumor suppressor genes. *Oncogene* 1995; 11: 2121-2126.
22. Vocke CD, Pozzatti RO, Bostwick DG, Florence CD, Jennings SB, Strup SE, Duray PH, Liotta LA, Emmert-Buck MR, Linehan WM: Analysis of 99 microdissected prostate carcinomas reveals a high frequency of allelic loss on chromosome 8p12-21. *Cancer Res* 1996; 56: 2411-2416.
23. Kerangueven F, Essioux L, Dib A, Noguchi T, Allione F, Geneix J, Longy M, Lidereau R, Eisinger F, Pebusque MJ, Jacquemier J, Bonaiti-Pellie C, Sobol H, Birnbaum D: Loss of heterozygosity and linkage analysis in breast carcinoma: indication for a putative third susceptibility gene on the short arm of chromosome 8. *Oncogene* 1995; 10: 1023-1026.
24. Yaremko ML, Wasylyshyn ML, Paulus KL, Michelassi F, Westbrook CA: Deletion mapping reveals two regions of chromosome 8 allele loss in colorectal carcinomas. *Genes Chromosomes Cancer* 1994; 10: 1-6.
25. Emi M, Fujiwara Y, Nakajima T, Tsuchiya E, Tsuda H, Hirohashi S, Maeda Y, Tsuruta K, Miyaki M, Nakamura Y: Frequent loss of heterozygosity for loci on chromosome 8p in hepatocellular carcinoma, colorectal cancer, and lung cancer. *Cancer Res* 1992; 52: 5368-5372.
26. Girard L, Zochbauer-Muller S, Virmani AK, Gazdar AF, Minna JD: Genome-wide allelotyping of lung cancer identifies new regions of allelic loss, differences between small cell lung cancer and non-small cell lung cancer, and loci clustering. *Cancer Res* 2000; 60: 4894-4906.
27. Wistuba II, Behrens C, Virmani AK, Milchgrub S, Syed S, Lam S, Mackay B, Minna JD, Gazdar AF: Allelic losses at chromosome 8p21-23 are early and frequent events in the pathogenesis of lung cancer. *Cancer Res* 1999; 59: 1973-1979.
28. El-Naggar AK, Coombes MM, Batsakis JG, Hong WK, Goepfert H, Kagan J: Localization of chromosome 8p regions involved in early tumorigenesis of oral and laryngeal squamous carcinoma. *Oncogene* 1998; 16: 2983-2987.
29. Kurimoto F, Gemma A, Hosoya Y, Seike M, Takenaka K, Uematsu K, Yoshimura A, Shibuya M, Kudoh S: Unchanged frequency of loss of heterozygosity and size of the deleted region at 8p21-23 during metastasis of lung cancer. *Int J Mol Med* 2001; 8 (1): 89-93.
30. Xu J, Zheng SL, Komiya A, Mychaleckyj JC, Isaacs SD, Hu JJ, Sterling D, Lange EM, Hawkins GA, Turner A, Ewing CM, Faith DA, Johnson JR, Suzuki H, Bujnovszky P, Wiley KE, DeMarzo AM, Bova GS, Chang B, Hall MC, McCullough DL, Partin AW, Kassabian VS, Carpten JD, Bailey-Wilson JE, Trent JM, Ohar J, Bleecker ER, Walsh PC, Isaacs WB, Meyers DA: Germline mutations and sequence variants of the macrophage scavenger receptor 1 gene are associated with prostate cancer risk. *Nat Genet* 2002; 32: 321-325.
31. Fong KM, Zimmerman PV, Smith PJ: Microsatellite instability and other molecular abnormalities in non-small cell lung cancer. *Cancer Res* 1995; 55: 28-30.
32. Mao L, Lee DJ, Tockman MS, Erozan YS, Askin F, Sidransky D: Microsatellite alterations as clonal markers for the detection of human cancer. *Proc Natl Acad Sci U S A* 1994; 91: 9871-9875.

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