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Down-regulation of *KAI1* Messenger RNA Expression Is Not Associated with Loss of Heterozygosity of the *KAI1* Gene Region in Lung Adenocarcinoma

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***KAI1*, a metastasis suppressor gene of prostate cancer, is located on human chromosome 11p11.2. Down-regulation of *KAI1* mRNA during tumor progression and metastasis has been reported for several kinds of cancer, but the mechanism of this down-regulation is not known. In the present study, our aim was to ascertain the relationship between down-regulation of *KAI1* mRNA expression and *KAI1* gene alterations in lung cancer. Forty-nine cases of adenocarcinoma of the lung were studied by reverse-transcriptase polymerase chain reaction (RT-PCR) assay of *KAI1* mRNA and by immunohistochemical detection of KAI1 protein. In addition, markers of the microsatellite loci D11S1344 and D11S1326 were used to investigate loss of heterozygosity (LOH) and replication errors (RERs) of the *KAI1* gene region. The RT-PCR assay showed that there was no correlation between *KAI1* mRNA expression and either the age of the patients or tumor size. By contrast, *KAI1* mRNA expression was significantly correlated with gender (P=0.047), metastasis to the lymph nodes or other organs (P=0.004), the histological grade of the tumor (P=0.036) and the pathological stage (P=0.049). Immunohistochemical staining showed that in one case without metastasis, loss of *KAI1* mRNA was associated with invasion of the stroma by KAI1 protein-negative cancer cells. The numbers of informative cases by microsatellite analysis were 14 (28.6%) of 49 at D11S1344 and 27 (55.1%) of 49 at D11S1326; none of 49 adenocarcinomas showed LOH or RERs at these loci. These results suggest that down-regulation of *KAI1* mRNA expression rarely if ever involves LOH or RERs of the *KAI1* gene region in primary lung adenocarcinoma.**

Key words: *KAI1* — Lung adenocarcinoma — RT-PCR — Immunohistochemistry — Microsatellite analysis

Lung cancer was the most lethal malignant neoplasm in Japanese males in 1993,[1)] and more than 33,000 male persons die each year from lung cancer. The most serious problem in the treatment of lung cancer is the poor prognosis after surgery, even when the disease stage is early; the 5-year survival rates are 67% for stage IA and 57% for stage IB.[2)] These data suggest that microinvasion or micrometastasis exists at the time of surgery and can not be controlled, so it is necessary to clarify the mechanism of invasion or metastasis.

Recently the *KAI1* gene (human chromosome 11p11.2) was identified as a metastasis suppressor gene of prostate cancer; decreased *KAI1* mRNA expression is involved in the progression of prostate cancers with metastatic ability.[3)] KAI1 protein is identical to CD82, consists of 267 amino acids, and is a member of the transmembrane 4 superfamily, which includes CD9,[4)] CD37,[5)] CD53,[6)] CD63[7)] and CD81.[8)] Members of this family are cell membrane glycoproteins containing a type III integral membrane structure with four transmembrane domains.[9)] The KAI1 protein also has one large extracellular hydrophilic domain with three potential N-glycosylation sites. These sites are consistent with the suggested role of metastasis suppression.[10)]

Reports about many cancer types, including lung cancer,[11, 12)] pancreatic cancer,[13)] breast cancer,[14)] bladder cancer[15)] and colon cancer,[16)] have pointed out that down-regulation of *KAI1* mRNA is related to tumor progression, but it is not clear whether this down-regulation involves DNA alterations of the *KAI1* region or not. In non-small cell lung cancer, low expression of *KAI1* mRNA and protein correlates with a poor prognosis,[11, 12)] but this correlation is significant only for adenocarcinoma and not for squamous cell carcinoma, so it seems that degrees of expression of *KAI1* mRNA and protein have different significance with regard to invasion or metastasis of carcinoma cells between these two histological types, although the reason for this is unclear.

The aim of the present study was to clarify the relationship between down-regulation of *KAI1* mRNA and the metastatic ability of lung adenocarcinomas, as well as to examine whether its down-regulation is associated with DNA alterations.

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MATERIALS AND METHODS

Clinical and pathological characteristics Tumor tissue sections from 49 Japanese patients with adenocarcinoma of the lung, 28 men and 21 women aged 31–80 years (mean, 64 years), who were treated at Hiroshima University Hospital, were obtained from the surgical file for the period from June 1991 to February 1998. None of the patients had received pre-operative adjuvant chemotherapy. The post-surgical pathological stage of each tumor was classified according to the international TNM classification.[17] There were 10 stage IA, 16 stage IB, 3 stage IIB, 16 stage IIIA, 3 stage IIIB and 1 stage IV tumors. The tumors were 1.4–13.0 cm (mean, 4.0 cm) in diameter and there were 16 well-differentiated, 22 moderately differentiated and 11 poorly differentiated tumors. Metastasis to the lymph nodes or other organs had occurred in 29 of the 49 cases.

Tissue samples One-half of each freshly resected tumor tissue had been frozen in OCT (optimal cutting temperature) compound (Miles Laboratories, Elkhart, IN) at −20°C at the time of surgery. At that time serial frozen sections with 5 μm thick had been cut on a cryostat and placed on 3-aminopropyl triethoxysilane (APS)-coated glass slides before storage at −80°C. The histological types of the carcinomas were confirmed by two pathologists independently using representative specimens of the tumors stained with hematoxylin and eosin. The other half of each tumor and its adjacent non-cancerous lung tissue, which had been stored whole at −80°C, were used for reverse-transcriptase polymerase chain reaction (RT-PCR) and microsatellite DNA analyses.

RT-PCR Total RNA was isolated from each tumor specimen and from 12 paired specimens of non-cancerous lung tissue by acid-guanidinium-phenol-chloroform extraction. Then the total RNA (1 μg from each specimen) was reverse transcribed to obtain cDNAs in a 20-μl reaction mixture containing 1.2 μM oligo$(dT)_{18}$ primer (Sigma Chemical Co., St. Louis, MO), each deoxynucleotide at 500 μM (Takara, Kyoto), 50 mM Tris-hydrochloride (pH 8.3), 75 mM KCl, 3 mM $NaCl_2$, 10 mM DTT, 20 units of RNase inhibitor (RNasin; Promega, Madison, WI) and 200 units of Moloney murine leukemia virus RNaseH RT (Superscript; Life Technologies, Inc., Gaithersburg, MD). The reaction mixture was incubated at 37°C for 60 min. PCR was carried out in a volume of 50 μl including a 1-μl aliquot of the cDNA reaction mixture, each primer at 0.5 μM, 0.25 mM deoxynucleotide triphosphate, 10 mM Tris-HCl buffer (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 2 units of Taq polymerase (Ampli-Taq Gold; Perkin-Elmer, Foster City, CA) using an automated temperature control system (PC-800; Astec, Fukuoka). Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) amplification was used as an internal control. To obtain reproducible quantitative data from the RT-PCR assay we chose the number of amplification cycles that gave linear amplification of the *KAI1* and *GAPDH* cDNAs. The PCR conditions for the *KAI1* and *GAPDH* cDNAs were 30 or 22 cycles respectively of denaturation at 94°C for 45 s, annealing at 62°C (*KAI1* cDNA) or 60°C (*GAPDH* cDNA) for 45 s, and extension at 72°C for 120 s. The PCR products were separated by electrophoresis on a 2% agarose gel and stained with 0.2% ethidium bromide. The intensity of each band was evaluated by a CCD (charge-coupled device) image sensor (Densitograph AE-6900-F; Atto, Tokyo) under UV illumination. Negative control reactions without cDNA were carried out for each primer set and no product was detected (data not shown).

The primer sequences for KAI1[3] were 5′-AGTCCTCCCTGCTGCTGTGTG-3′ (sense) and 5′-TCAGTCAGGGTGGGCAAGAGG-3′ (antisense). This primer pair amplifies a 1031-bp fragment (nucleotides 65–1095). The primer sequences for *GAPDH* were 5′-CTCAGACACCATGGGGAAGGTGA-3′ (sense) and 5′-ATGATCTTGAGGCTGTTGTCATA-3′ (antisense).

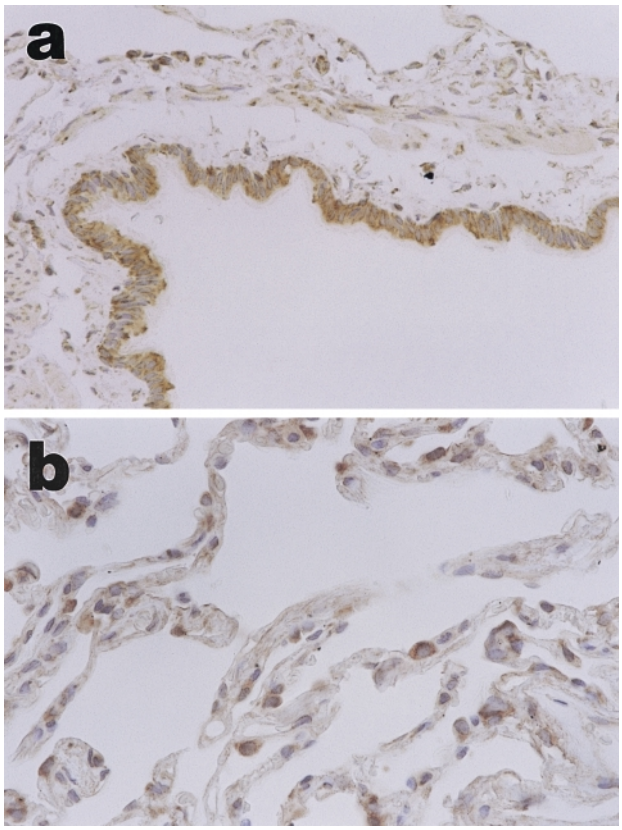

Fig. 1. Immunohistochemical staining of KAI1 protein (hematoxylin counterstain). Normal bronchial epithelium adjacent to cancer region (a) (×200) and normal alveolar cells adjacent to cancer region (b) (×200) were positive.

Specimen classification based on mRNA levels Here the *KAI1* mRNA level is presented as the density of the *KAI1* cDNA band divided by that of the *GAPDH* band in the same gel. All of the non-cancerous lung tissues we examined had higher *KAI1* mRNA levels than the tumor tissues and it has been shown previously, by northern blot analysis, that lung tissue expresses more *KAI1* mRNA than any other human tissue.[3] So we concluded that *KAI1* mRNA was conserved in our non-cancerous lung tissue specimens and we used the mean *KAI1* mRNA level of the non-cancerous lung tissues as a control value. The level of *KAI1* mRNA in each tumor was expressed relative to this control value. Ratios over 0.4 were considered to indicate conserved *KAI1* mRNA expression, whereas ratios less than 0.4 were considered to indicate reduced *KAI1* mRNA expression in the tumor.

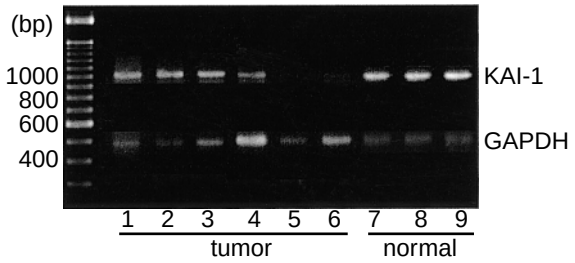

Fig. 2. Electrophoresis of RT-PCR amplified *KAI1* cDNA and *GAPDH* cDNA (internal PCR control) of the same specimens. Lanes 1–3 show primary adenocarcinomas of the lung with conserved *KAI1* mRNA expression. Lanes 4–6 show primary adenocarcinomas of the lung with reduced *KAI1* mRNA expression. Lanes 7–9 were non-cancerous lung tissues (positive control of *KAI1* mRNA expression).

Immunohistochemical analysis The archival frozen sections were fixed in acetone at –20°C for 7 min and then in 4% paraformaldehyde at 4°C for 7 min. Staining was performed by the avidin-biotin-peroxidase complex method using a commercial kit (Histofine SAB-PO kit; Nichirei, Tokyo). To inhibit endogenous peroxidase activity, the sections were incubated at room temperature for 30 min in 0.3% H_2O_2 in absolute methanol. Incubation with the anti-

Table I. Correlation between *KAI1* mRNA Expression and Clinicopathological Factors in Lung Adenocarcinoma

Clinicopathological factors	No. of cases	*KAI1* mRNA expression		*P*
		+	–	
Age at surgery (year)				
<60	14	10 (71%)	4 (29%)	0.310
60≤, <70	22	10 (45%)	12 (55%)	
70≤	13	7 (54%)	6 (46%)	
Gender				
male	28	12 (43%)	16 (57%)	0.047
female	21	15 (71%)	6 (29%)	
Tumor size (cm)				
≤3	21	11 (52%)	10 (48%)	0.779
3<	28	16 (57%)	12 (43%)	
Metastasis to lymph nodes or other organs				
–	29	21 (72%)	8 (28%)	0.004
+	20	6 (30%)	14 (70%)	
Histological types				
well differentiated	16	13 (81%)	3 (19%)	0.036
moderately differentiated	22	9 (41%)	13 (59%)	
poorly differentiated	11	5 (45%)	6 (55%)	
Stage				
I A	10	8 (80%)	2 (20%)	0.049
I B	16	11 (69%)	5 (31%)	
II A	0	— —	— —	
II B	3	2 (67%)	1 (33%)	
III A	16	4 (25%)	12 (75%)	
III B	3	2 (67%)	1 (33%)	
IV	1	0 (0%)	1 (100%)	

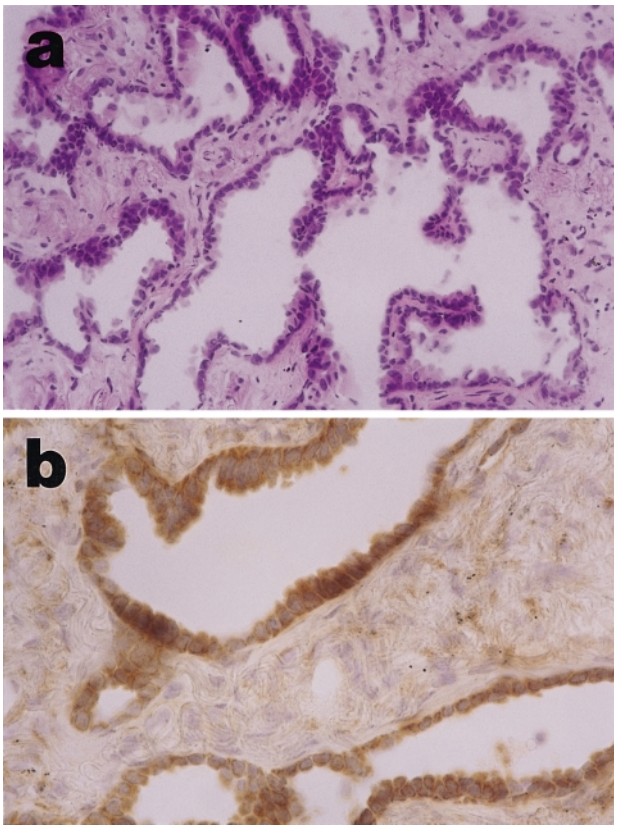

Fig. 3. Microscopic view of well differentiated adenocarcinoma of the lung. Serial sections were stained with hematoxylin and eosin (a) (×100) and anti-KAI1 antibody (b) (×200). The tumor showed the highest expression of KAI1 protein.

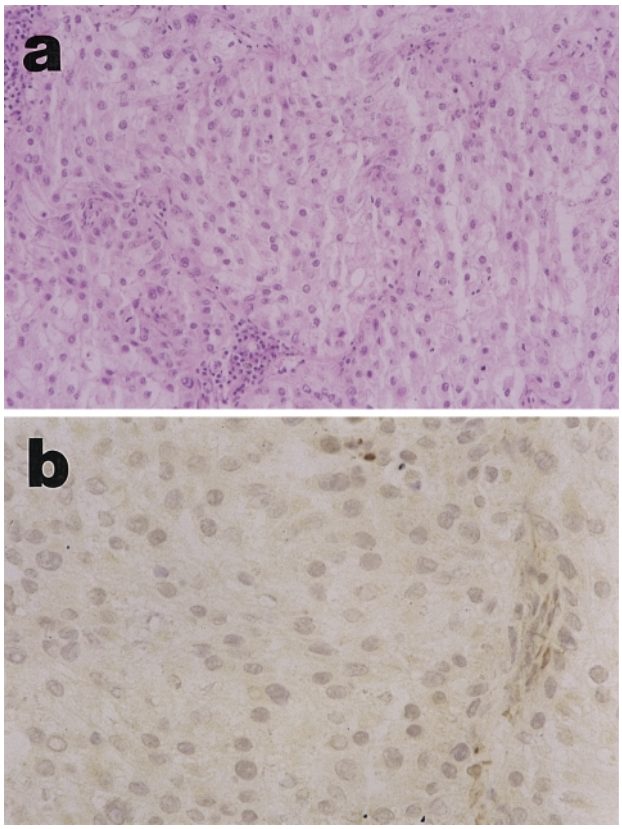

Fig. 4. Microscopic view of poorly differentiated adenocarcinoma of the lung. Serial sections were stained with hematoxylin and eosin (a) (×100) and anti-KAI1 antibody (b) (×200). The tumor showed no expression of KAI1 protein.

KAI1 rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was done at 4°C overnight. The sections were counterstained with hematoxylin. In the negative control, the anti-KAI1 antibody was replaced with phosphate-buffered saline (PBS).

Specimen classification based on protein levels KAI1 protein was expressed on the cell membrane or in the cytoplasm of various cell types. Besides the adenocarcinoma cells, bronchial and bronchiolar epithelial cells and alveolar cells most clearly expressed KAI1 (Fig. 1), whereas vascular endothelial cells, smooth muscle cells of the vascular wall and fibroblasts showed uneven expression. When the staining intensity of the adenocarcinoma cells was similar to or stronger than that of the non-cancerous bronchiolar epithelial or alveolar cells, cellular KAI1 protein expression was considered to be positive, otherwise it was considered to be negative. Moreover, when the frequency of KAI1-positive cells was over 50% in a tumor specimen, the KAI1 protein expression of the tumor was considered to be conserved, otherwise it was considered to be reduced. These judgments were made by two pathologists independently.

Microsatellite analysis of loss of heterozygosity (LOH) or replication error (RER) DNA was isolated from each tumor tissue and its adjacent non-cancerous lung tissue by phenol-chloroform extraction. The polymorphic microsatellite markers D11S1326 and D11S1344 are located near the *KAI1* gene.[18] The primer sequences used were 5′-TGCCAAGAACAGCAAAA-3′ (CA strand) and 5′-GGGGTTCAAAATAAACAAAA-3′ (GT strand) for D11S1326 and 5′-CCCTGAACTTCTGCATTCAC-3′ (CA strand) and 5′-GCGCCTGGCTTGTACATATA-3′ (GT strand) for D11S1344. PCR was carried out in a volume of 50 μl including 50 ng of genomic DNA, each primer at 0.5 μM, 0.25 mM deoxynucleotide triphosphate, 10 mM Tris-HCl buffer (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2 units of Taq polymerase and 5 μCi [α-^{32}P]dCTP. PCR was for 27 cycles of denaturation at 94°C for 40 s, annealing at 56°C for 60 s, and extension at 72°C for 120 s. The PCR products were separated by electrophoresis on a 6% polyacryl-

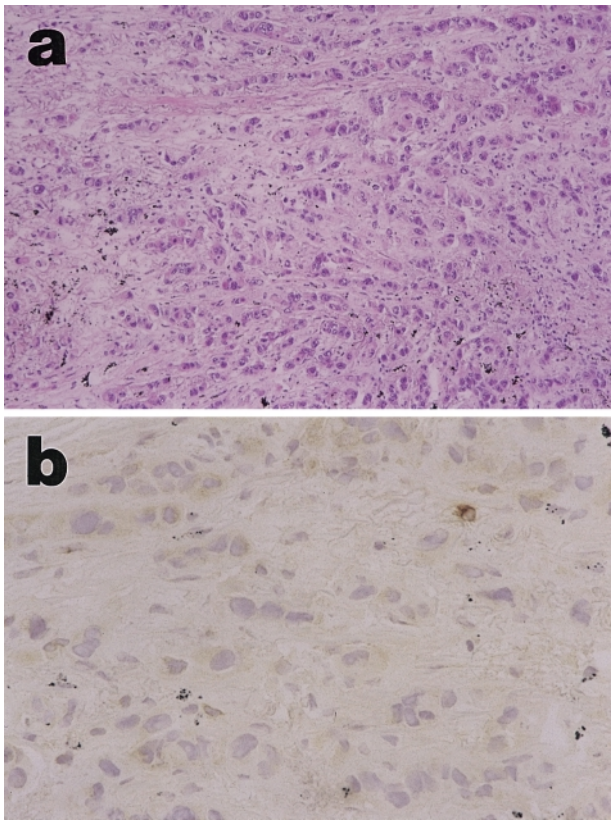

Fig. 5. Microscopic view of case 6, which had no metastasis clinicopathologically and had reduced *KAI1* mRNA expression by RT-PCR assay. Serial sections were stained with hematoxylin and eosin (a) (×100) and anti-KAI1 antibody (b) (×200). Adenocarcinoma cells that expressed no KAI1 protein had invaded the stroma.

amide gel. Autoradiography was for 1 day. Negative control reactions without DNA were carried out for each primer set and no product was detected (data not shown).

The radioactivity of the PCR products was compared between tumor and non-cancerous DNA in each case. We measured the intensity of the band amplified from each allele and used the intensity ratio of two alleles in non-cancerous DNA as the control of heterozygosity. We diagnosed LOH only when the observed intensity of one allele in the tumor DNA was less than 50% of expected intensity, which was calculated from the control.

Statistical analysis Statistical evaluations were performed using the χ^2 test. Correlations were regarded as significant when the *P*-value was less than 0.05.

RESULTS

Correlation between *KAI1* mRNA expression and clinicopathological factors The quantitative RT-PCR assay showed that 27 of 49 (55.1%) lung adenocarcinomas had conserved *KAI1* mRNA expression (Fig. 2). There was no correlation between *KAI1* mRNA expression and patients' age or tumor size, though *KAI1* mRNA expression did significantly correlate with gender (P=0.047), metastasis to the lymph nodes or other organs (P=0.004), the histological grade of the tumor (P=0.036) and the pathological stage (P=0.049) (Table I). The tumors of female patients showed conserved *KAI1* mRNA expression more often than those of the male patients and *KAI1* mRNA expression was also conserved more frequently in cases without metastasis compared to cases with metastasis. Furthermore, the well-differentiated adenocarcinomas had a higher frequency of conserved *KAI1* mRNA expression than the moderately differentiated or poorly differentiated tumors. Similarly, there was a tendency for less advanced tumors to show conserved *KAI1* expression more often than advanced cases.

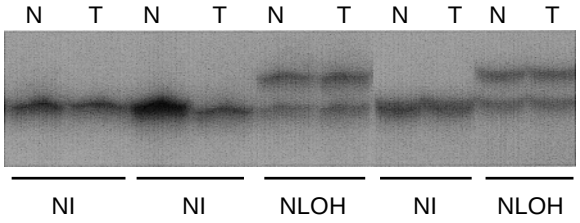

Fig. 6. Microsatellite analysis at the D11S1344 locus in lung adenocarcinoma. N, DNA from non-cancerous tissues; T, DNA from primary adenocarcinomas; NI, not informative; NLOH, informative (retention of heterozygosity), LOH negative.

Table II. Microsatellite Analysis of LOH or RER of 11p in Lung Adenocarcinoma

Loci	No. of cases with LOH or RER/No. of informative cases	
	KAI1 mRNA expression	
	+	–
D11S1344	0/4	0/10
D11S1326	0/10	0/17

Immunohistochemical staining Of 49 adenocarcinomas, 24 (49%) showed conserved KAI1 protein expression (Figs. 3 and 4). These results were generally consistent with the RT-PCR data. Case 6 was unusual because there was no metastasis to the lymph nodes or other organs even though both *KAI1* mRNA expression and KAI1 protein expression were reduced. However, we observed histologically that KAI1 protein-negative adenocarcinoma cells had invaded the stroma in this specimen (Fig. 5).

Microsatellite analysis The numbers of informative cases were 14 of 49 (28.6%) examined cases at D11S1344 and 27 of 49 (55.1%) at D11S1326 (Fig. 6). All informative

cases were grouped based on the conservation or reduction in *KAI1* mRNA expression. None of the adenocarcinomas showed LOH or RERs in any case (Table II).

DISCUSSION

Some kinds of cancer show down-regulation of *KAI1* mRNA during the progression of tumors with metastatic ability, but the mechanism of this down-regulation has not been identified. Moreover, the involvement of *KAI1* gene alterations in prostate cancer is controversial. Kawana *et al*. observed LOH or RERs at D11S1344 in 7 of 10 (70%) informative metastatic lesions and in 3 of 9 (33%) informative primary prostate tumors.[18] However, Dong *et al.* reported that LOH was never seen at the same locus (D11S1344) in 34 primary prostate tumors and 12 metastatic lesions.[19] Hence, the relevance of DNA alterations of the *KAI1* gene region to metastasis is far from clear. Therefore we conducted microsatellite analysis in lung adenocarcinomas by using the markers D11S1344 and D11S1326, which are close to the *KAI1* locus.

First, RT-PCR analysis was performed to identify each case as having conserved or reduced *KAI1* mRNA expression in the tumor. We observed that *KAI1* mRNA expression in the tumor was reduced more often in men than in women. This difference may be due to smoking, though the smoking index was not investigated in this study. Sex hormones also may influence *KAI1* expression, but no evidence for this has been reported to date. We observed a strong negative correlation between metastasis and *KAI1* mRNA expression; 70% of the carcinomas with metastasis had reduced *KAI1* mRNA expression. This observation is in agreement with previous reports about several organic cancers.[11–13, 15, 16, 19, 20] In the present series, we observed that metastasis occurred more frequently in men than in women ($P=0.045$). It is possible that the significant correlation between gender and *KAI1* mRNA expression might be related to the frequency of metastasis. It is unclear why 30% of the carcinomas with metastasis had normal *KAI1* mRNA expression, although there are several possibilities to consider. First, these tumors may have decreased levels of proteins that up-regulate the function of KAI1, or increased levels of proteins that down-regulate the function of KAI1. Second, the three potential N-glycosylation sites of the KAI1 protein, which are thought to be associated with its role in metastasis suppression, may be dysfunctional.[10] By contrast, 28% of the carcinomas without metastasis showed reduced *KAI1* mRNA expression. Interestingly, in case 6, adenocarcinoma cells that expressed no KAI1 protein had invaded the stroma but not the lymph nodes or other organs. Hence, when KAI1 protein expression is reduced in cancer cells, microinvasion may occur even when there is no spread to distant sites. So far, the precise role of KAI1 is not understood, but like the other members of the transmembrane 4 superfamily, KAI1 protein may play a role in cell-cell interactions, which are important in invasion and metastasis. The immunohistochemical staining result of case 6 is consistent with this hypothesis. Indeed, in a recent study on breast carcinoma cell lines, low expression of *KAI1* mRNA was associated with high metastatic propensity and invasive ability.[14] It has also been reported that after gene transfer to induce the reexpression of KAI1 protein in highly metastatic rat prostate cancer cells, colon cancer cells and melanoma cells, these cells showed reduced invasiveness and motility.[3, 16, 21] These reports suggest that KAI1 protein is an invasion-suppressor glycoprotein. We observed that well-differentiated adenocarcinomas had conserved *KAI1* mRNA expression more frequently than moderately or poorly differentiated adenocarcinomas. Although the factors that determine the degree of differentiation have not been identified so far, KAI1 protein may influence the differentiation of cancer cells. We analyzed the relationship between the histological grade of the tumor and metastasis, but found no correlation ($P=0.481$). Thus, histological grade and metastasis were considered to be independent factors for *KAI1* mRNA expression.

Our microsatellite analysis detected no LOH or RERs in any of 49 primary lung tumors at D11S1344 and D11S1326. By contrast, Kawana *et al.*[18] observed LOH or RERs at D11S1344 in 7 of 10 (70%) informative metastatic lesions, in 3 of 9 (33%) informative primary prostate tumors and in 1 of 12 (8%) informative clinically localized prostate tumors. However, there are some technical differences between our study and theirs. First, they grouped their specimens into localized tumors, primary carcinomas with metastasis and metastatic lesions, whereas we grouped our specimens based on the conservation or reduction in *KAI1* mRNA expression because microinvasion may occur even if there is no metastasis. Second, we did not analyze metastatic lesions. Usually, the cancer cells of a metastatic lesion are at a more advanced stage than those of the primary tumor. Indeed, Higashiyama *et al*. reported that the KAI1 protein expression levels in metastatic lesions were diminished compared with levels of expression in the primary lung lesions.[12] Consequently, the results of our study suggest that down-regulation of *KAI1* mRNA expression rarely involves loss of heterozygosity or replication errors near the *KAI1* gene in primary lung adenocarcinoma.

Recently, it was reported that several CpG-rich islands are present in the 5′ promoter region of the *KAI1* gene, so methylation may be associated with the down-regulation of *KAI1* mRNA.[19, 22] It has also been suggested that the loss of p53 function may lead to down-regulation of *KAI1* gene expression.[23] In the light of these findings further investigations are necessary to identify the mechanisms of down-regulation of *KAI1*.

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REFERENCES

1) Health and Welfare Statistics Association. *Annu. Stat. Rep. Natl. Health*, **44**, 433–434 (1997).
2) Mountain, C. F. Revisions in the international system for staging lung cancer. *Chest*, **111**, 1710–1717 (1997).
3) Dong, J.-T., Lamb, P. W., Rinker-Schaeffer, C. W., Yukanovic, J., Ichikawa, T., Isaacs, J. T. and Barrett, J. C. KAI-1, a metastatic suppressor gene for prostatic cancer on human chromosome 11p11.2. *Science*, **268**, 884–886 (1995).
4) Miyake, M., Koyama, M., Seno, M. and Ikeyama, S. Identification of the motility-related protein (MRP-1), recognized by monoclonal antibody M31-15, which inhibits cell motility. *J. Exp. Med.*, **174**, 1347–1354 (1991).
5) Classon, B. J., Williams, A. F., Willis, A. C., Seed, B. and Stamenkovic, I. The primary structure of the human leukocyte antigen CD37, a species homologue of the rat MRC OX-44 antigen. *J. Exp. Med.*, **169**, 1497–1502 (1989).
6) Amiot, M. Identification and analysis of cDNA clones encoding CD53: a panleukocyte antigen related to membrane transport proteins. *J. Immunol.*, **145**, 4322–4325 (1990).
7) Hotta, H., Ross, A. H., Huebner, K., Isobe, M., Wendeborn, S., Chao, M. V., Ricciardi, R. P., Tsujimoto, Y., Croce, C. M. and Koprowski, H. Molecular cloning and characterization of an antigen associated with early stages of melanoma tumor progression. *Cancer Res.*, **48**, 2955–2962 (1988).
8) Oren, R., Takahashi, S., Doss, C., Levy, R. and Levy, S. TAPA-1, the target of an antiproliferative antibody, defines a new family of transmembrane proteins. *Mol. Cell. Biol.*, **10**, 4007–4015 (1990).
9) Wright, M. D. and Tomlinson, M. G. The ins and outs of the transmembrane-4 superfamily. *Immunol. Today*, **15**, 588–594 (1995).
10) Hakomori, S. Aberrant glycosylation in tumors and tumor-associated carbohydrate antigens. *Adv. Cancer Res.*, **52**, 257–331 (1989).
11) Adachi, M., Taki, T., Ieki, Y., Huang, C. L., Higashiyama, M. and Miyake, M. Correlation of KAI1/CD82 gene expression with good prognosis in patients with non-small cell lung cancer. *Cancer Res.*, **56**, 1751–1755 (1996).
12) Higashiyama, M., Kodama, K., Yokouchi, H., Takami, K., Adachi, M., Taki, T., Ishiguro, S., Nakamori, S., Yoshie, O. and Miyake, M. KAI1/CD82 expression in nonsmall cell lung carcinoma is a novel, favorable prognostic factor. *Cancer*, **83**, 466–474 (1998).
13) Guo, X., Friess, H., Graber, H. U., Kashiwagi, M., Zimmermann, A., Korc, M. and Buchler, M. W. KAI1 expression is up-regulated in early pancreatic cancer and decreased in the presence of metastasis. *Cancer Res.*, **56**, 4876–4880 (1996).
14) Yang, X., Welch, D. R., Phillips, K. K., Weissman, B. E. and Wei, L. L. KAI1, a putative marker for metastatic potential in human breast cancer. *Cancer Lett.*, **119**, 149–155 (1997).
15) Yu, Y., Yang, J.-L., Markovic, B., Jackson, P., Yardley, G., Barrett, J. C. and Russell, P. J. Loss of KAI1 messenger RNA expression in both high-grade and invasive human bladder cancers. *Clin. Cancer Res.*, **3**, 1056–1059 (1997).
16) Takaoka, A., Hinoda, Y., Satoh, S., Adachi, Y., Itoh, F., Adachi, M. and Imai, K. Suppression of invasive properties of colon cancer cells by a metastasis suppressor KAI1 gene. *Oncogene*, **16**, 1443–1453 (1998).
17) International Union Against Cancer (UICC). "TNM Classification of Malignant Tumors," 5th Ed., ed. L. H. Sobin and C. H. Wittekind (1997). John Wiley & Sons, Inc., New York.
18) Kawana, Y., Komiya, A., Ueda, T., Nihei, N., Kuramochi, H., Suzuki, H., Yatani, R., Imai, T., Dong, J.-T., Imai, T., Yoshie, O., Barrett, J. C., Isaacs, J. T., Shimazaki, J., Ito, H. and Ichikawa, T. Location of KAI1 on the short arm of human chromosome 11 and frequency of allelic loss in advanced human prostate cancer. *Prostate*, **32**, 205–213 (1997).
19) Dong, J.-T., Suzuki, H., Pin, S. S., Bova, G. S., Schaalken, J. A., Isaacs, W. B., Barrett, J. C. and Isaacs, J. T. Down-regulation of the KAI1 metastasis suppressor gene during the progression of human prostatic cancer infrequently involves gene mutation or allelic loss. *Cancer Res.*, **56**, 4387–4390 (1996).
20) Ueda, T., Ichikawa, T., Tamaru, J., Mikata, A., Akakura, K., Akimoto, S., Imai, T., Yoshie, O., Shiraishi, T., Yatani, R., Ito, H. and Shimazaki, J. Expression of the KAI1 protein in benign prostatic hyperplasia and prostatic cancer. *Am. J. Pathol.*, **149**, 1435–1440 (1996).
21) Takaoka, A., Hinoda, Y., Sato, S., Itoh, F., Adachi, M., Hareyama, M. and Imai, K. Reduced invasive and metastatic potentials of *KAI1*-transfected melanoma cells. *Jpn. J. Cancer Res.*, **89**, 397–404 (1998).
22) Dong, J.-T., Isaacs, W. B., Barret, J. C. and Isaacs, J. T. Genomic organization of the human KAI1 metastasis-suppressor gene. *Genomics*, **41**, 25–32 (1997).
23) Mashimo, T., Watabe, M., Hirota, S., Hosobe, S., Miura, K., Tegtmeyer, P. J., Rinker-Shaeffer, C. W. and Watabe, K. The expression of the KAI1 gene, a tumor metastasis suppressor, is directly activated by p53. *Proc. Natl. Acad. Sci. USA*, **95**, 11307–11311 (1998).