American Journal of Pathology, Vol. 149, No. 5, November 1996

Short Communication

Expression of the KAI1 Protein in Benign Prostatic Hyperplasia and Prostate Cancer

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The KAI1 gene, recently identified as a metastatic suppressor gene for prostate cancer, was cloned and was revealed to be identical to the C33/IA4/R2/4R9 gene. The expression of KAI1 protein was examined immunohistochemically in the tissues from 14 cases of benign prostatic hyperplasia and 46 cases of prostate cancer using mouse monoclonal anti-human C33 antibody. In benign prostatic hyperplasia tissues, KAI1 protein was uniformly expressed in the glandular cell membrane at cell-to-cell borders. The KAI1 protein in the tissues of untreated prostate cancer was also located at similar sites to those of benign prostatic hyperplasia, but the percentage of strongly positive cancer cells was correlated inversely to the Gleason pattern ($P < 0.0001$, one-way analysis of variance). There was also a statistically inverse correlation between the percentage of KAI1-positive cancer cells and the clinical stage ($\chi^2 = 9.6$; $P = 0.0081$). In 4 cancer death cases relapsed from endocrine therapy, KAI1 protein was not stained in either primary or metastatic foci. These results indicate that the expression of KAI1 protein correlates to tumor characteristics in prostate cancer. (Am J Pathol 1996, 149:1435–1440)

Many genetic events have been shown to be associated with the development of solid cancers, and it is now generally accepted that transformation of normal tissue to malignancy is accompanied by an accumulation of genetic changes in oncogenes and tumor suppressor genes.[1] Previous cytogenetic and molecular biological studies in prostate cancer revealed that high rates of allelic deletions occur on chromosomes 7q, 8p, 10, 16q, and 18q.[2–8]

Recently, Dong et al[9] isolated a metastatic suppressor gene for prostate cancer from the human chromosome region 11p11.2 and designated it as KAI1. The KAI1 gene suppressed metastasis when it was introduced into a highly metastatic subline from Dunning R3327 rat prostate cancer cells. The KAI1 gene was cloned and, surprisingly, the sequence of the complementary DNA (cDNA) was identical to that of C33 that was reported in human lymphocytes.[10,11] The gene encodes an open reading frame of a 267-amino-acid sequence that was a type III integral membrane protein of 29.6 kd with four putative transmembrane domains and three putative *N*-glycosylation sites.[10,11] The mouse monoclonal antibody against the C33 antigen has been established.[10]

The present study was undertaken to examine the expression of KAI1 protein in benign hyperplastic and cancerous human prostatic tissues immunohistochemically using the mouse monoclonal anti-C33 antibody.[10,11]

Supported by grants-in-aid from the Ministry of Education, Science, and Culture (06281103 and 07671709) and the Ministry of Health and Welfare (5–10, New 10-year Strategy for Cancer Control), Japan.

Accepted for publication July 15, 1996.

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Materials and Methods

Tissue Samples

Tissues from patients with adenocarcinoma of the prostate who were treated at Chiba University Hospital between July 1994 and December 1995 were obtained from needle biopsy (42 cases) before treatment. Tissues from 4 cases, who initially showed response to endocrine therapy but thereafter relapsed and died from cancer were obtained at autopsy 2 to 6 hours postmortem from prostate and metastatic foci (lymph node, 2 cases; liver, 1 case; and lung, 1 case). Tissues from 14 patients with benign prostatic hyperplasia (BPH) were obtained by either retropubic prostatectomy or needle biopsy. After removal, tissues were immediately embedded in optimal cutting temperature compound (Miles, Elkhart, IN) and stored at −80°C. Sections (6 μm) were cut on a cryostat, mounted on aminopropyltriethoxysilane-coated slides, air dried, and used for staining. One section was stained with hematoxylin and eosin and confirmed histologically. Tumor grade and clinical stage were classified according to the Gleason method and the Jewett-Whitmore system.[12,13]

Immunohistochemical Staining of KAI1 Protein

The sections were fixed with acetone for 10 minutes at 4°C and air dried. All subsequent steps were performed at room temperature. The sections were incubated with methanol containing 0.3% hydrogen peroxide for 30 minutes to block endogenous peroxidase activity. After treatment with phosphate-buffered saline (PBS) containing 5% normal rabbit serum for 30 minutes, the sections were incubated with mouse monoclonal anti-human C33 antibody at a dilution of 1:1000 in PBS for 2 hours in a moist chamber.[10,11] After treatment with biotinylated rabbit anti-mouse immunoglobulin (Dako, Glostrup, Denmark) diluted 1:500 in PBS for 30 minutes, the sections were incubated with biotin-streptavidin-peroxidase complex (Dako) for 30 minutes, followed by a 3,3′-diaminobenzidine tetrahydrochloride and hydrogen peroxide mixture. The sections were counterstained with hematoxylin, dehydrated in graded ethanol, cleared in xylene, and mounted. As negative control, nonimmunized purified rat IgG2a (Zymed Laboratories, San Francisco, CA) was used instead of the anti-human C33 antibody.

Staining intensity in the cancer cells was estimated as positive when it appeared to be similar to that of glandular cells of BPH. When relationships between the expression of KAI1 protein and the Gleason pattern were examined, the percentage of positively stained cells was calculated in 500 cells showing the same Gleason pattern. When the relationship between the expression of KAI1 protein and clinical stage was estimated, the staining pattern of the KAI1 expression was classified as abundant (51 to 100% of cells stained), decreased (5 to 50% of cells stained), or negative (0 to 4% of cells stained). Statistical significance was calculated by a one-way analysis of variance or the χ^2 test.

Results

In BPH specimens, KAI1 protein was expressed uniformly in the cellular membrane of glandular epithelial cells, and especially at areas of cell-to-cell borders (Figure 1, A and B). KAI1 protein was not detected in the nucleus or cytoplasm.

The expression of KAI1 protein was variable in the 42 biopsy specimens obtained from untreated prostate cancer. Expression of KAI1 protein in the cancer cells was also observed on the membrane at cell-to-cell borders, being similar in pattern and intensity to those observed in BPH specimens (Figure 1, C and D). The percentage of KAI1-positive cells in the tissues of BPH and untreated prostate cancer is shown in Figure 2. In cancer tissues with Gleason patterns 2 and 3, averages of 70 and 67% of the cancer cells showed positive expression of KAI1 protein, respectively. However, in less differentiated tumors (Gleason pattern 4), a lower rate of positively stained cells was noticed. In these specimens, the immunostaining pattern along cell-to-cell borders was not continuous (Figure 1E). Most cancer tissues with Gleason pattern 5 consisted of cells that showed a negative expression of KAI1 protein (Figure 1F). In the stromal cells of the BPH and cancer tissues, fibroblastic cells were positively stained. As shown in Figure 2, a significant inverse correlation existed between the expression of KAI1 protein and Gleason pattern ($P < 0.0001$, one-way analysis of variance). There was also a statistically significant inverse correlation between the expression of KAI1 protein and the clinical stage ($\chi^2 = 9.6$; $P = 0.0081$; Table 1).

In the four cancer death cases relapsed from endocrine therapy, both primary prostate cancer and metastatic cancer tissues showed a Gleason score of 10 and a negative expression of KAI1 protein.

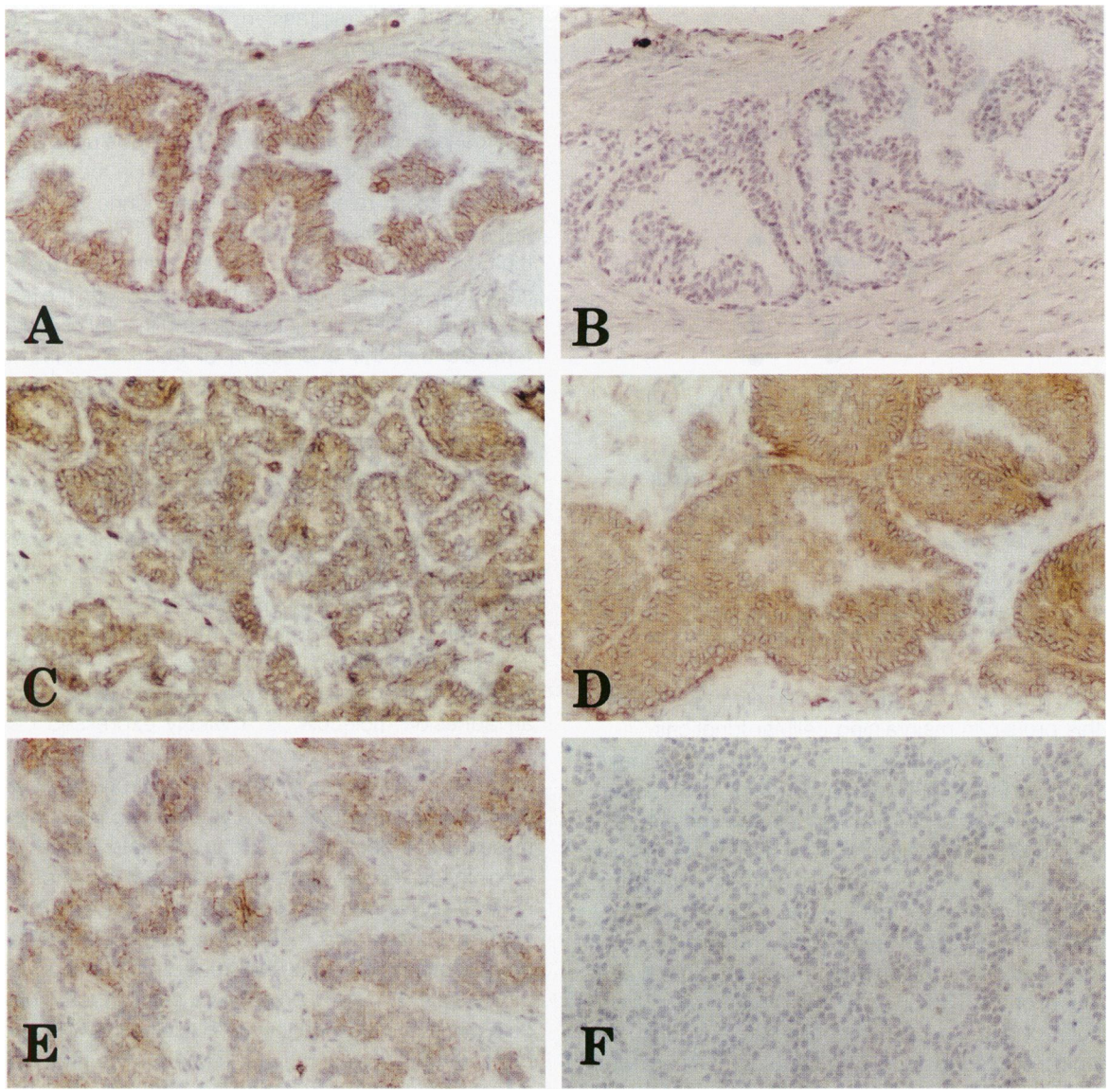

Figure 1. *Immunohistochemical staining of KAI1 protein in tissues of BPH* (A *and* B) *and prostate cancer* (C *to* F) *using monoclonal anti-C33 antibody with counterstaining (hematoxylin). Magnification, ×180.* A: *KAI1 expression of glandular and stromal cells.* B: *Negative control in the same tissue as* A. C: *Cancer cells of Gleason pattern 2 tumor showing positive expression of KAI1 protein at the cell-to-cell border.* D: *Cancer cells of Gleason pattern 3 tumor. Most cells showed positive expression of KAI1 protein.* E: *Cancer cells of Gleason pattern 4 tumor. Positive cells were scattered with loss of continuity in the cell-to-cell border.* F: *Negative staining of the tumor cells of the Gleason pattern 5 tumor.*

Discussion

The KAI1 gene is identical to the cDNA obtained from various human leukocytes, and this cDNA was named variously in several laboratories as C33, IA4, R2, or 4F9. [11,14–16] The product of this gene is assumed to be a surface marker of human lymphocytes[11,14–16] and is classified as CD82. Originally, the C33 antigen was identified by monoclonal antibody inhibitory to syncytium formation induced by human T cell leukemia virus type I.[10] The monoclonal antibody against C33 antigen was used in the present study. The C33 protein was shown to localize on the cellular membrane and is thought to be a member of the transmembrane 4 superfamily (TM4SF).[11] Most TM4SF molecules have a common characteristic, ie, that they contain four hydrophobic, presumably membrane-spanning segments and a single major, presumably extracellular, and usually *N*-glycosylated loop.[17] Although the biological functions of the TM4SF have not yet been sufficiently clarified, recent investigations have suggested that these molecules are involved in the regulation of cell growth and cell-to-cell adhesion.[18–20] In this context, the present study has shown the presence of KAI1

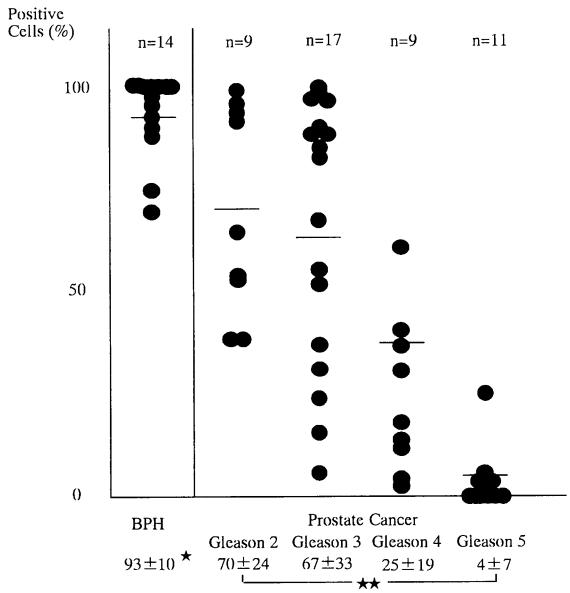

Figure 2. *Percentage of KAI1-protein-positive cells in the glandular part from BPH and the cancer cells of untreated prostate cancer. ★, mean ± SD; ★★, P < 0.0001 by one-way analysis of variance.*

protein in BPH, which suggests some important roles of this protein in maintaining cellular functions.

Some molecules belonging to the TM4SF have been reported to be related to tumor progression and metastasis.[9,21,22] The melanoma-associated antigen ME491/CD63, a member of the TM4SF, is not detected in normal tissues but is strongly expressed in the early stage of melanoma. This expression, however, is weaker or negative in the late stage of human malignant melanoma.[23] MRP-1/CD9, also a member of the TM4SF, was found as a cell surface antigen of non-T acute lymphoblastic leukemia, developing B lymphocytes, and platelets.[18] There was a significant inverse correlation between the MRP-1/CD9 expression in primary cancer tissues and the lymph node status of breast cancer.[22] Moreover, the MRP-1/CD9 expression in the metastatic lymph node was lower than that in primary breast carcinoma foci based on immunohistochemical and reverse transcriptase polymerase chain reaction analyses.[22] The KAI1 gene is a member of the TM4SF, and the present study showed that less differentiated cancer cells diminish its expression. This may indicate that KAI1 protein represents some tumor characteristics of prostate cancer. The presence of this protein correlates with the clinicopathological features of prostate cancer, as is shown in the present study. Both primary and metastatic cancer tissues in the cancer death cases relapsed from endocrine therapy showed a negative expression of KAI1 protein, and thus cancer cells in the endocrine therapy-relapsed state may acquire malignant potential with the escape from cell-to-cell interactions. Recently, it was reported that the expression of the KAI1 gene in non-small-cell lung cancer correlated with tumor grade, nodal status, and pathological features, indicating that KAI1 was a prognostic factor.[24] This result is similar to the tendency observed in prostate cancer.

It has been reported that decreased expression of E-cadherin is correlated with tumor grade, tumor stage, and poor prognosis in prostate cancer.[25,26] The nm23 expression has been reported to be inversely correlated with the development of metastasis in prostate cancer.[27] CD44, recently reported as an adhesion molecule, is a widely expressed cell surface glycoprotein functioning in both cell-to-substrate and cell-to-cell interaction.[28] Expressions of CD44 and its variant forms are related to properties in some cell lines from human prostate cancer.[29] From these reports, it may be said that various cell surface proteins reveal biological tumor activities including invasion and metastasis, as these membranous proteins influence the adhesive properties of tumor cells. The KAI1 protein is a cell surface molecule that may influence the cell-to-cell adhesion, and therefore this protein may be related to the metastasis-inhibiting activity reported by Dong et al.[9]

Many factors have been reported to correlate with the malignant potential of prostate cancer. Clinical stage, tumor grade, response to endocrine therapy, the extent of disease at initial bone scan, and the expression of androgen receptor have been proposed as prognostic factors of prostate cancer.[12,30–33] The presence of surface proteins, including KAI1, may represent new prognostic factors.

Table 1. *Relationship between KAI1 Immunohistochemical Staining Pattern and Clinical Stage in Untreated Prostate Cancer*

Clinical stage	Staining pattern			Statistical significance
	Abundant	Decreased	Negative	
B–C	14	7	1	$\chi^2 = 9.6$
D2	5	7	8	$P = 0.0081$

The staining pattern was categorized according to the percentage of positively stained cells: abundant (51 to 100%), decreased (5 to 50%), negative (0 to 4%). Statistical significance was estimated by the χ^2 test.

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