Loss of KAI1 Messenger RNA Expression in Both High-Grade and Invasive Human Bladder Cancers

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ABSTRACT

The molecular mechanisms responsible for metastasis are not fully understood. Recently, expression of the KAI1 gene on human chromosome 11p11.2 was found to be down-regulated in metastatic prostate cancer cell lines compared with normal human prostate, suggesting that KAI1 may be a metastasis suppressor gene. The aim of this study was to investigate whether there is reduced expression of KAI1 in late-stage bladder cancer. Sixty-six paraffin-embedded bladder tissue sections were analyzed for KAI1 mRNA by in situ hybridization. Nineteen of these were from patients with in situ bladder cancer. The costs of publication of this article were defrayed in part by the The abbreviations used are: TCC, transitional cell carcinoma; TM4, the specimens of normal bladder (11 of 11; 100%), inflammatory bladder (5 of 8; 63%), and noninvasive papillary TCCs of grades 1 and 2 (15 of 24; 63%), compared to grade 3 papillary TCCs (1 of 7; 14%) or invasive TCCs (1 of 16; 6%). The differences in expression between local and invasive disease were statistically significant (P ≤ 0.01, x^2 test). Our results suggest that down-regulation of KAI1 mRNA is significantly associated with invasive bladder cancer and that KAI1 may represent an invasion/metastasis suppressor gene in bladder cancer.

INTRODUCTION

In the United States, there are approximately 50,000 new cases and 10,000 deaths from bladder cancer each year (1). It is the fifth most common cancer in men and the eighth in women (2). Similar figures occur in Australia, where bladder cancer is responsible for 2% (women) or 4% (men) of all new malignant neoplasms and 1.5% (women) or 2.4% (men) of cancer deaths in New South Wales (3). TCC^2 accounts for more than 90% of bladder tumors. Between 70 and 80% of bladder cancers are identified as superficial lesions, and 20% are invasive at first diagnosis (1). The invasive tumors have a poor prognosis, with a patient overall 5-year mortality rate of almost 50% (4). Low-grade superficial tumors tend to be more stable, with 93% of patients surviving more than 20 years (5). Bladder cancers are frequently multifocal and have a high recurrence rate even after apparently successful treatment by standard measures such as diathermy; radiotherapy; chemotherapy; or, ultimately, radical cystectomy. Muscle-invasive and metastatic diseases are the main causes of mortality for patients with bladder cancer.

The carcinogenesis of bladder cancer is a multistage phenomenon in which a variety of genetic alterations can be segregated into early and late events. Early events would be responsible for the initial transformation of urothelial cells, with late events providing these transformed clones with the means to invade or metastasize (6). A variety of chromosomal abnormalities are associated with the development of TCC and may correlate with prognosis, including nonrandom changes of chromosomes 1, 3, 5, 6, 7, 8, 9, 10, 11, 13, 17, and 18 (reviewed in Refs. 6 and 7). Loss of heterozygosity of chromosome 9 has been documented in a broad range of bladder cancer, irrespective of stage, whereas genetic alterations of chromosomes 3p, 5q, 11p, 17p, and 18q appear to be late events. Changes in a variety of oncogene/suppressor genes have been described in bladder cancer, including ras, myc, erbB-2, Rh, and p53 (7).

From the above studies, it has been suggested that there are two molecular pathways in the development of bladder cancer. In one, loss of heterozygosity of chromosome 9 leads to the development of noninvasive papillary cancer, whereas p53 mutations are associated with the development of carcinoma in situ and dysplasia (8). Accumulating evidence has suggested that chromosome 11p deletions are a frequent occurrence in tumor invasion and metastasis (9–11).

Recently, a tumor metastasis suppressor gene, KAI1, has been isolated from human chromosome 11p11.2 (12). This gene encodes a 2.4-kb mRNA and a polypeptide predicted to be a transmembrane protein of 267 amino acids belonging to the TM4 superfamily, so called because they span the plasma membrane four times (13). Other members of this family include CD9, CD37, CD53, CD63, CD81, and CD82/C331R2 (13–15). In contrast to the limited expression of other members of the TM4 family, KAI1 is expressed in all tissues thus far examined and is most abundant in prostate, lung, kidney, bone marrow, and placenta, with high levels also observed in breast, pancreas, skeletal muscle, and thymus (12). Although their functions are unknown, the localization of the TM4 superfamily proteins to the cell membrane suggests that they may be involved in cell-cell or cell-extracellular matrix interactions, both of which would be important in metastasis.

An initial study showed that the KAI1 gene suppressed metastasis when introduced into rat AT6.1 prostate cancer cells (12). Furthermore, down-regulation of KAI1 protein levels has
and probed with a tissues were obtained from Clontech (multiple Northern blot) and Lane 3).
molecular sizes of the KAII and β-actin mRNAs are indicated on the left of the figure. Lanes 1–8 represent spleen (Lane 1), thymus (Lane 2), prostate (Lane 3), testes (Lane 4), ovary (Lane 5), small intestine (Lane 6), colon (mucosal lining; Lane 7), and peripheral blood leukocyte (Lane 8).

been found in metastatic prostate cancer samples compared to normal prostate (16). Similar results have been observed in both lung cancer and breast cancer cell lines (17, 18). In addition, correlation of KAII gene expression with good prognosis was found in patients with non-small cell lung cancer (19). These studies suggest that KAII may act as a suppressor of metastases in a wide range of cancers. The situation may be more complex, however, because in pancreatic cancer, there is up-regulation of KAII in early disease followed by a decrease in the presence of metastases (20). At present, the potential role of KAII in the progression of bladder cancer is unknown.

The purpose of this study was to determine whether down-regulation of KAII gene expression occurs in bladder cancer. Retrospective samples of normal bladder tissue, different grades of TCC (21), and invasive bladder cancer were used. Antibodies to KAII that are effective on paraffin-embedded tissue are not yet available. To overcome this limitation, in this study we used a KAII cDNA probe to examine mRNA levels by ISH.

MATERIALS AND METHODS

Archival Tissues. Bladder tissue sections were provided by AMRAD Proprietary Ltd. Fresh tissues were all fixed in a standard fashion in formaldehyde solution for more than 24 h, embedded in paraffin, and cut at 5 μm. Among 66 sections, 47 were papillary TCCs of grades 1, 2, or 3, and 16 contained invasive tumors. Nineteen normal or inflammatory bladder tissues were taken by needle biopsy from patients with no histological evidence of bladder cancer; six of these showed chronic bladder inflammation. This study was approved by the Research Ethics Committee of the South Eastern Sydney Area Health Service.

Preparation of a KAII Probe. A 1.03-kb [nucleotide residues 64–1094 of KAII cDNA sequence (12)] KAII probe was amplified from plasmid pCMV-KAII (donated kindly by Dr. J.-T. Dong, John Hopkins Oncology Center, Baltimore, MD) by PCR using a 5’ primer, 5’-AGTTCCTCCCTGCTCTGCT-3’, and 3’ primer, 5’-CTCTCTTGCCCCACCTGTA-3’. Reaction conditions used an initial cycle of 5 min at 96°C, 1 min at 55°C, and 2 min at 72°C followed by 25 cycles of 1 min and 30 s at 96°C, 1 min at 60°C, and 2 min at 72°C. The final cycle was 10 min at 72°C. The amplified DNA product was checked on a 1% agarose gel, and a single band at 1.03 kb was visualized with ethidium bromide. The DNA product was recovered and purified by standard methods (22).

Northern Blotting. The levels of KAII mRNA expression in eight different human tissues, including normal prostate, were examined using a human multiple tissue Northern blot (MTN Blot; Clontech Laboratories, Inc., Palo Alto, CA). Hybridization using a 32P-labeled KAII PCR product was performed as described (22). To verify equal loadings of RNA, the MTN Blot was also probed with a 32P-labeled β-actin cDNA (Clontech).

ISH. A modified ISH procedure (23) was performed to detect KAII mRNA expression in paraffin-embedded tissue sections. Briefly, dried slides were covered by a strip of paraffin containing a section-shaped hole in the center. In addition to the KAII probe, a β-actin cDNA probe was used as an internal positive control (24), and pBR322 was used as a negative control. A no-probe control was used to rule out possible effects of endogenous biotin and endogenous alkaline phosphatase. Probes were labeled with photobiotin using the BRESATEC (Adelaide, South Australia) procedure (25). Detection of bound probes were then performed using a commercial ISH Detection Kit (K600; DAKO A/S, Glostrup, Denmark). The substrates we used were naphthol AS-MX phosphate and fast red TR salt (Sigma Chemical Co., Sydney, NSW, Australia), giving a red color to positive signals, which was not influenced by counterstaining with Mayer’s hematoxylin. All sections were first screened by ISH using β-actin. Only those sections that were β-actin positive were chosen for analysis of KAII staining. Red staining in urothelial cells was considered a positive signal for KAII staining. The intensity of staining was reported as weak or strong, and the percentage of cells showing staining was also assessed. Negative results were those in which urothelial cells remained blue.

Statistical Analysis. A standard χ2 analysis was performed to assess the significance of the differences between the tissues of normal bladder, inflammatory bladder, and low-grade papillary TCC and the tissues of high-grade, invasive papillary TCC.

RESULTS

The specificity of our KAII PCR product was first verified by Northern blot hybridization (Fig. 1). Consistent with previous results (12), a single hybridizing species of mRNA at 2.4 kb was detected in all eight human tissues. As reported previously,

Table 1  Positive expression of KAII mRNA in bladder tissues

<table>
<thead>
<tr>
<th>Bladder tissue</th>
<th>KAII mRNA No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>11 of 11 (100%)</td>
</tr>
<tr>
<td>Inflammatory</td>
<td>5 of 8 (63%)</td>
</tr>
<tr>
<td>Papillary TCC</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>4 of 6 (67%)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>11 of 18 (61%)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>1 of 7 (14%)</td>
</tr>
<tr>
<td>Papillary TCC, invasive</td>
<td>1 of 16 (6%)</td>
</tr>
</tbody>
</table>

*χ2 test, P < 0.0001 between (t + s) and (t' + s').
levels of KAI1 expression varied between different tissues. The highest KAI1 expression was observed in prostate, thymus, and small intestine, with lower levels identified in spleen, ovary, and peripheral blood leukocyte. The lowest levels were detected in colon (mucosal lining) and testis. These variations in KAI1 mRNA levels were not due to variations in RNA loading, as indicated by the expression of β-actin mRNA, also shown in Fig. 1.

Positive expression of KAI1 mRNA was found in all groups of bladder tissues but not in all cases in each group, as shown in Table 1. It was highly expressed in normal tissues (11 of 11; 100%), inflammatory tissues (5 of 8; 63%), and low-
grade papillary TCCs (4 of 6 (67%) for grade 1 and 11 of 18 (61%) for grade 2) but disappeared in most of the grade 3 papillary and invasive TCCs (Fig. 2A–E). Only 5–10% of tumor cells were stained weakly in the two positive specimens from high-grade and invasive bladder cancer, whereas in other non-invasive papillary TCC, normal tissues, and inflammatory tissues, strong staining of an average of 50–75% of urothelial cells was observed. The distribution of KAI1 mRNA was mainly in the cytoplasm and on the membrane of urothelium. Some positive staining was seen in muscle cells but not in stromal cells.

Thus, in the bladder, significantly reduced expression of KAI1 mRNA was associated with papillary TCC (16 of 47) compared with normal or inflamed tissue (16 of 19; x-squared test, P = 0.001), and this appeared to be due to loss of expression in invasive disease (1 of 16; 6%) compared to noninvasive papillary TCC (16 of 31 (52%); P = 0.006). There appeared to be reduced expression of KAI1 in grade 3 papillary TCCs (1 of 7; 14%) compared with tumors of lower grade (15 of 24; 63%), but this difference was not statistically significant (P = 0.06); however, higher expression was observed in both grade 1 (4 of 6) and grade 2 (11 of 18) than in grade 3 papillary TCCs (1 of 7; P = 0.01 and P = 0.006, respectively). No statistically significant difference in KAI1 mRNA expression was observed between invasive carcinomas (1 of 16) and grade 3 TCCs (1 of 7; P = 0.861), although the numbers examined were small.

**DISCUSSION**

We have shown that levels of the KAI1 mRNA appear to be related to the progression of bladder cancer based on our finding that down-regulation of this gene correlates with increased grade and stage.

Because there are no other published reports concerning KAI1 expression in bladder cancer, we have compared our study with those done in other cancers. Expression of the KAI1 gene was reported to be capable of inhibiting metastasis but not tumorigenesis when expressed in rat prostatic cancer cells (12), and its expression was significantly decreased in prostate tissue biopsies from metastatic compared with normal samples (12, 16). These initial studies suggested that KAI1 might be a metastatic suppressor gene specific for prostate cancer. However, subsequent studies have shown a significant correlation between decreased expression of KAI1 mRNA and poor prognosis in patients with non-small cell lung adenocarcinoma (19). In addition, reduced levels of KAI1 protein have been observed in metastatic breast cancer cell lines (17, 18), which were restored by transfer of chromosome 11 into the cells (18). The increased levels of KAI1 protein correlated with a reduction in metastatic potential. Taken together with our data, these findings suggest that the KAI1 protein may be a more general suppressor of cancer metastasis. However, KAI1 may also play a role in the early stages of certain cancers, because in pancreatic cancer, its expression was up-regulated in early cancer and then decreased in the presence of metastases (20).

In the current study, KAI1 mRNA expression was found to be significantly down-regulated in high-grade tumors (grade 3 papillary TCCs) and more especially in invasive bladder cancers when compared to normal, inflammatory, and low-grade TCCs examined. These results support the premise that partial or complete loss of KAI1 function is associated with the progression of bladder cancer.

The KAI1 gene is located on chromosome 11p (12), which also contains loci associated with other oncogenes and tumor suppressor genes (26). Abnormalities of 11p, including deletions (9–11) and numerical changes, such as trisomy and tetrasomy (26, 27), have been detected in late-stage or aggressive bladder cancers. Although it is possible that some of these alterations might contribute to the down-regulation of KAI1 mRNA, a recent study of KAI1 gene mutation and allelic loss in prostatic cancer failed to reveal any relationship between such changes and KAI1 expression (16). Due to the unavailability of further tissue, it has not been possible to explore any relationship between loss of heterozygosity on 11p and a decrease in KAI1 mRNA expression in the current study, but this will be the subject of a future prospective study.

The product of KAI1 is a type III integral membrane protein with four transmembrane domains, which belongs to a superfamily of cell surface membrane glycoproteins. The localization of KAI1 protein on the membrane of prostate epithelial cells (16) suggests that it could be involved in cellular interactions, possibly by mediating signaling between cells and their environment, affecting cell movement and differentiation. Support for this notion has come from a recent study, which demonstrated a link between the integrin cell adhesion molecules, TGF-β, and phosphatidylinositol 4-kinase (28).

In conclusion, our results suggest that down-regulation of KAI1 mRNA is significantly associated with invasive bladder cancer and that KAI1 may represent an invasion/metastasis suppressor gene in bladder carcinomas. Loss of KAI1 expression in bladder cancer biopsies may indicate the need for more aggressive therapy.

**REFERENCES**


Loss of KAI1 messenger RNA expression in both high-grade and invasive human bladder cancers.

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