International Journal of Urology (2004) **11**, 74–82

Original Article

Decreased expression of *KAI1* metastasis suppressor gene is a recurrence predictor in primary pTa and pT1 urothelial bladder carcinoma

JING-SHI SU,[1] KIMINOBU ARIMA,[1] MARIKO HASEGAWA,[1] OMAR E. FRANCO,[1] YOSHIKI UMEDA,[1] MAKOTO YANAGAWA,[1] YOSHIKI SUGIMURA[1] AND JUICHI KAWAMURA[2]

[1]*Department of Urology, Faculty of Medicine, Mie University, Tsu, Mie and* [2]*Ijinkai Takeda General Hospital, Kyoto, Japan*

Abstract

Objective: To examine the expression of the *KAI1* metastasis suppressor gene and to evaluate its relationship with tumor recurrence in primary pTa and pT1 urothelial bladder carcinoma.

Methods: Samples were obtained from 87 patients after transurethral resection (TUR). Tumor stage and grade were reviewed in 33 patients with pTa and in 54 patients with pT1, with a mean follow-up time of 47.4 ± 30.1 months. The *KAI1* protein immunohistochemical assay was performed. Prognosis was analyzed using the Kaplan–Meier method and Cox's proportional hazards model. Correlation between *KAI1* expression and recurrence according to each clinicopathological factor was comparatively evaluated using the chi-squared test.

Results: Decreased expression of *KAI1* protein failed to reach statistical significance for stage ($P = 0.25$) or morphology of tumor stem ($P = 0.19$), but it was significantly related to tumor size ($P = 0.016$). The recurrence-free 5-year survival rates of the group with decreased *KAI1* expression was 69.7%, which was significantly higher than the 22.2% for the *KAI1*-positive group ($P < 0.0001$). In univariate and multivariate analyses, decreased expression of *KAI1* protein, stage pT1, tumor size >3 cm and sessile tumors were independent prognosis factors of recurrence. Despite the lower recurrence rate expected by considering only the clinicopathological factors, decreased *KAI1* expression was able to identify the group with a high risk of recurrence.

Conclusions: Downregulated *KAI1* expression in bladder tumors tends to relate to stage and morphology of the tumor stem and was significantly correlated to tumor size. Decreased expression of *KAI1* was associated with the degree of invasiveness and progression of the cancer and was an independent prognostic factor of recurrence in primary pTa and pT1 urothelial bladder carcinoma.

Key words clinicopathological factors, *KAI1* expression, pTa and pT1 urothelial bladder carcinoma, recurrence.

Introduction

Bladder carcinoma is the second most common malignancy encountered by urologists; approximately 70–80% of patients with bladder carcinoma present with pTa and pT1 urothelial bladder carcinomas,[1] which can be managed with transurethral resection (TUR) alone or with intravesical therapy. However, recurrence occurs in more than half of all cases of pTa and pT1 urothelial bladder carcinoma within 5 years after TUR, and the majority of patients who progress eventually succumb to their disease.[2] Tumor recurrence is the most important problem in the treatment of pTa and pT1 urothelial bladder carcinoma. Different causes have been proposed to explain why tumor recurrence may appear, including the persistence of residual tumor due to incomplete resection, tumor may be present but not visible at the time of TUR, cancer cells from the primary tumor may

Correspondence: Jing-Shi Su, Department of Urology, Faculty of Medicine, Mie University, 2-174 Edobashi, Tsu-shi, Mie 514-8504, Japan.
Email: sujinshi@clin.medic.mie-u.ac.jp
Received 17 March 2003; accepted 19 August 2003.

have transplanted to other parts of the urothelium and/or it can be attributable to the continual insult of the carcinogenic process.[3] Several recurrence risk factors have been reported, such as grade, stage, tumor morphology, tumor size and tumor number.[4–6] Among these risk factors, the number of tumors and tumor size are considered the most important prognostic factors related to recurrence.[7,8] Access to molecular biology analysis has permitted as to understand those factors involved in bladder cancer, such as chromosome 9,[9] the *p53* suppressor gene[10] and *Ki-67* antigen.[11] Also, the detection of abnormal genes has become possible using clinicopathology. Recent studies have shown that multifocal bladder cancer and tumor recurrence have a monoclonal origin and suggest that tumor cells have either an intraepithelial spread or are transplanted directly to the bladder mucosa after TUR.[12–15]

The *KAI1* metastasis suppressor gene has been examined in many cancers, including bladder carcinoma specimens and cell lines,[16–20] and its expression has been related to tumor invasiveness, metastases, growth of metastatic tumors,[21] cell motility and adhesion.[22–24] It has been reported that decreased *KAI1* expression appears at an early phase of tumor progression[25–28] and is a predictor of recurrence in breast cancer and small cell lung cancer.[20,29] Thus, whether examination of *KAI1* gene expression could contribute to predicting the recurrence of cancer in primary pTa and pT1 bladder urothelial carcinoma is unknown. Using immunohistochemical staining, we examined *KAI1* gene expression, evaluated the correlation between *KAI1* expression and clinicopathological factors and also studied its effect on tumor recurrence.

Methods

Clinical characteristics of patients

Tumor specimens were obtained from 87 patients (72 males and 15 females) with primary pTa and pT1 bladder urothelial (transitional cell) carcinoma who were treated by TUR of the bladder tumor at Mie University Hospital. The mean age of patients was 64 ± 13 years (range 34–91 years). Patients were followed up for a mean of 47.4 ± 30.1 months (range 4–78 months). After TUR, five patients underwent intravesical Bacillus Calmette–Guérin (BCG) instillation treatment and 77 patients received intravesical anticancer drug (mitomycin C and cytosine arabinoside or adriamycin) treatment. Tumor stage and grade were reviewed and determined according to the *General Rule for Clinical and Pathologic Studies on Bladder Cancer* of the Japanese Urological Association and the Japanese Society of Pathology,[30] which is based on the TNM classification[31] and World Health Organization criteria.[32] Recurrent time was calculated from the time of the initial TUR-bladder tumor (Bt) until the first tumor recurrence had been confirmed by cystoscopy. These patients did not show progress defined as the presence of muscle invasion, metastasis.[33] The clinicopathological characteristics of the patients are given in Table 1.

Immunohistochemical assays

Immunohistochemical staining was performed using the streptavidin–biotin (SAB) method. Tissue sections were obtained from formalin-fixed, paraffin-embedded samples and mounted on aminopropyltriethoxysilane-coated glass slides (DAKO, Kyoto, Japan). Tissue sections (3 μm) were deparaffinized and rehydrated. Endogenous peroxide was blocked by exposure to 0.3% hydrogen peroxide for 15 min and washed twice with phosphate-buffered saline (PBS). Subsequently, sections were placed in citric acid buffer (10 mmol/L, pH 6.0), followed by heating in a microwave oven (500 W) for five successive periods of 3 min, necessary for antigen activation. After heating, sections were allowed to cool in citrate buffer for 20 min at room temperature and were washed four times with PBS. To prevent non-specific binding, sections were blocked with a super-block (ScyTek stain kit; ScyTek Laboratories, West Logan, UT, USA) for 8 min at room temperature and washed with PBS. Sections were incubated overnight at 4°C with polyclonal serum to *KAI1* (C-16) from Santa Cruz Biotechnology (Santa Cruz, CA, USA; dilution 1 : 100), as described previously,[19] and washed four times with PBS. Subsequently, samples were incubated with biotinylated link antibody (ScyTek stain kit; ScyTek Laboratories) for 20 min at room temperature and washed four times with PBS. Sections were incubated with streptavidin/horseradish peroxidase (HRP) label (ScyTek stain kit; ScyTek Laboratories) for 20 min at room temperature and washed four times with PBS. After the above reaction ended, the peroxidase reaction was performed using a solution of 3,3′-diaminobenzidine (ScyTek stain kit; ScyTek Laboratories) as the chromogen substrate for 5 min. Finally, slides were lightly counterstained with hematoxylin and then observed.

In each specimen, endothelial and lymphocyte cells in tumor stroma were used as internal positive controls[27] and a normal serum at the same concentration of the primary antibody was used as a negative control. When staining intensity on the cell membrane and cytoplasm appeared to be similar to that of endothelial and

Table 1 Clinical characteristics of patients and association between decreased *KAI1* expression and clinicopathological factors in primary pTa and pT1 urothelial bladder carcinomas

Prognostic factors	Total (%)	Expression of *KAI1* (%)		*P*-value
		Positive	Decreased	
Histology				
Grade 1	23 (26.4)	16 (70)	7 (30)	0.78
Grade 2	56 (64.4)	33 (59)	23 (41)	
Grade 3	8 (9.2)	5 (62)	3 (38)	
Pathology				
Stage pTa	33 (37.9)	23 (70)	10 (30)	0.25
Stage pT1	54 (62.1)	31 (57)	23 (43)	
Morphology				
Papillary	75 (86.2)	48 (64)	27 (36)	0.35
Non-Papillary	12 (13.8)	6 (50)	6 (50)	
Pedunculated	60 (69.0)	40 (67)	20 (33)	0.19
Sessile	27 (31.0)	14 (52)	13 (48)	
Size (cm)				
<1	21 (24.1)	15 (71)	6 (29)	0.016
1–3	49 (56.3)	33 (67)	16 (33)	
>3	12 (13.8)	6 (50)	6 (50)	
Unidentified	5 (5.8)	0	5 (100)	
No. tumors				
1	46 (52.9)	29 (63)	17 (37)	0.12
2–4	25 (28.7)	14 (56)	11 (44)	
>5	10 (11.5)	9 (90)	1 (10)	
Unidentified	6 (6.9)	2 (33)	4 (67)	

lymphocyte cells, it was estimated to be strong positive (2+) or moderately positive (1+). When staining intensity appeared to be similar to the negative control, it was estimated to be negative (–), if staining intensity appeared to be between positive (1+) and negative (–), it was estimated to have a weaker staining (+/–).

The staining pattern of *KAI1* expression was classified as: (i) moderate (or positive) when tumor cell membranes and cytoplasm showed uniform and moderate staining or when tumor tissue staining was not uniform in intensity but more than 50% of tumor cells were stained; (ii) decreased if 5–50% of tumor cells were stained; and (iii) negative if less than 5% of tumor cells were stained for *KAI1* within the tumor tissue. As a staining contrast for the expression of *KAI1* protein in pTa and pT1 urothelial bladder carcinoma, tissue sections from 17 cases with non-malignant 'normal' adjacent urothelial and from 32 patients who underwent cystectomy with muscle-invasive bladder cancers (stage pT2–4) were evaluated at the same time.

Statistical analysis

Correlation between *KAI1* expression and clinicopathological factors (grade, stage, morphology, tumor size and number of tumors) and an association between decreased expression of *KAI1* protein and tumor recurrence according to each clinicopathological factor were evaluated comparatively by the chi-squared test. The recurrence rate for decreased *KAI1* expression and each clinicopathological factor was calculated based on the Kaplan–Meier method and log-rank test. To identify the risk factors for recurrence, we performed a multivariate analysis using Cox's proportion hazard model. These calculations were performed using StatView 5.0 software (SAS Institute, Cary, NC, USA) and $P < 0.05$ was considered statistically significant.

Results

Expression of KAI1 protein and clinicopathological factors

Staining was moderate or strong and uniformly spread in all 17 cases of 'normal' adjacent urothelial tissue of the bladder (Fig. 1a). In pTa and pT1 bladder urothelial carcinoma, the staining intensity was lower than for normal epithelial cells and ranged from uniform intense to negative staining or exhibited a heterogeneous staining pattern that has been described previously.[19] Fifty-four cases (62.1%) were classified as moderate expression (or positive; Fig. 1b) and 33 cases (37.9%) had decreased expression (Fig. 1c,d), including 29 cases

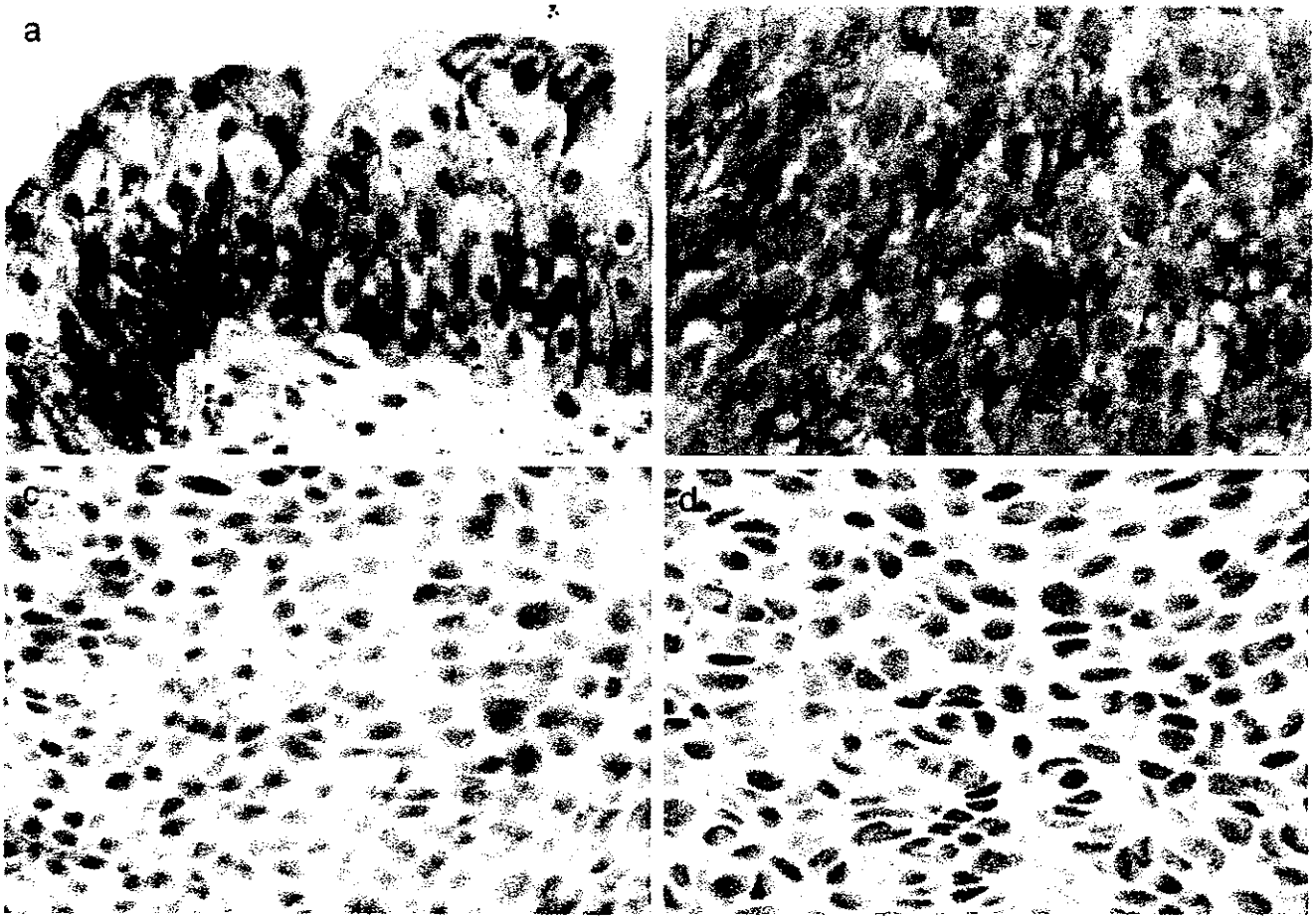

Fig. 1. (a) The 'normal' adjacent urothelial tissue of bladder carcinoma exhibited a uniform, moderate to strong staining of cell membranes and cytoplasm (original magnification ×400). (b) Moderate (or positive) *KAI1* expression. Tumor cells showed a uniform and moderately stained membrane and cytoplasm (original magnification ×400.) (c,d) Decreased (or negative) *KAI1* expression. Cells exhibited a non-uniform or heterogeneous staining (original magnification ×400.)

with less than 50% of stained tumor cells and four cases with no staining within the tumor tissue. In multiple tumors, a total of 116 tumors were investigated and 41 tumors (35.3%) were recorded as having the poorest staining pattern, exhibiting most staining at the surface of the tumor, secondly at fragments or heterogeneously in fragments (Fig. 1c) and at the base of tumors; these different staining patterns were also seen in large tumors (size >3 cm). In invasive bladder cancers, only three cases (9.4%) were classified as moderately stained and expression of *KAI1* protein was significantly lower ($P < 0.0001$) compared with the 62.1% found in pTa and pT1 tumors.

The correlation between the expression of *KAI1* protein and clinicopathological factors in pTa and pT1 urothelial bladder carcinoma is summarized in Table 1. There was no significant difference in the expression of *KAI1* protein in relation to tumor grade; seven of 23 grade 1 tumors (30%), 23 of 56 grade 2 tumors (41%) and three of eight grade 3 tumors (38%) showed decreased expression of *KAI1* protein. Decreased expression of *KAI1* protein was more common in stage pT1 tumors (43%) compared with stage pTa (30%). However, this difference failed to reach statistical significance ($P = 0.25$). Decreased expression of *KAI1* protein also showed a weak relationship with the morphology and number of tumors, but this correlation was not statistically significant ($P > 0.05$). Decreased expression of *KAI1* protein was significantly related to tumor size ($P = 0.016$).

Decreased KAI1 expression and tumor recurrence

In the present study, the recurrence rate after TUR was 25.3, 36.8 and 40.2% for 1, 3 and 5 years, respectively.

When recurrence factors were analyzed with *KAI1* and clinicopathological factors, the recurrence-free survival rates at 5 years were significantly related to the expression of *KAI1* protein (Fig. 2; $P < 0.0001$), stage, morphology of the tumor stem and tumor size (Table 2; $P = 0.0158$, $P = 0.007$ and $P = 0.0001$, respectively). Although there was a trend towards a higher recurrence rate in multiple tumors (two to four tumors), the difference was not statistically significant ($P = 0.3234$). Furthermore, in cases of more than five tumors, the recurrence-free survival rate at 5 years was lower (31.4%) than for cases with single tumors and, although the cause for this is uncertain, patients with multiple tumors also had lower *KAI1* expression (10%; Table 1). The results of multivariate analysis concur with those of univariate analysis, namely that decreased *KAI1* expression, stage pT1, sessile tumors and tumor size >3 cm are independent prognostic factors of tumor recurrence (Table 3; $P = 0.0013$, $P = 0.031$, $P = 0.0026$ and $P = 0.014$, respectively). Clinical material with a homogeneous tumor phenotype and consistent management are more relevant for testing new markers. We comparatively evaluated differences in expression of *KAI1* protein between the recurrence and non-recurrence groups according to each clinicopathological factor. Despite the low recurrence risk in univariate analysis for low grade, stage pTa, papillary tumors, tumor diameter <3 cm and single tumors (Table 2), when decreased *KAI1* expression was used, these factors also indicated an increased risk of recurrence (Table 4). These results show that decreased KAI1 expression can identify patients with a

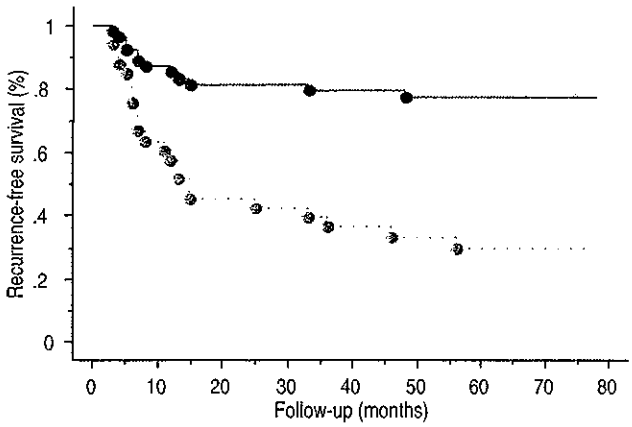

Fig. 2. Kaplan–Meier method for determining tumor recurrence-free survival rates of pTa and pT1 urothelial bladder carcinoma patients with either decreased or positive *KAI1* expression. (·····), decreased *KAI1* expression ($n = 33$); (——), positive *KAI1* expression ($n = 54$; $P < 0.0001$).

Table 2 Five-year recurrence-free survival rates according to each clinicopathologic factors in primary pTa and pT1 urothelial bladder carcinomas

Prognostic factors	Probability of recurrence (%)			*P*-value
	1 year	3 years	5 years	Log-rank test
Histology				
Grade 1	5 (21.7)	9 (39.1)	9 (39.1)	NS
Grade 2	15 (26.8)	20 (35.7)	22 (39.6)	
Grade 3	2 (25.0)	3 (37.5)	4 (68.8)	
Pathology				
Stage pTa	5 (15.2)	8 (24.2)	8 (24.2)	0.0158
Stage pT1	17 (31.5)	24 (44.4)	27 (51.3)	
Morphology				
Papillary	21 (28.0)	28 (37.3)	30 (40.3)	NS
Non-Papillary	1 (8.3)	4 (33.3)	5 (42.9)	
Pedunculated	14 (23.3)	16 (26.7)	18 (30.4)	0.007
Sessile	8 (29.6)	16 (59.3)	17 (63.3)	
Size (cm)				
<1	3 (14.3)	6 (28.6)	6 (28.6)	0.0001
1–3	11 (22.4)	13 (26.5)	15 (31.0)	
>3	7 (58.3)	9 (75.0)	10 (87.5)	
Unidentified	1 (20.0)	4 (80.0)	4 (80.0)	
No. tumors				
1	10 (21.7)	14 (30.4)	16 (35.2)	NS
2–4	7 (28.0)	13 (52.0)	13 (52.0)	
>5	2 (20.0)	2 (20.0)	3 (31.4)	
Unidentified	3 (50.0)	3 (50.0)	3 (50.0)	

Table 3 Independent prognostic indicators for recurrence selected by multivariate analysis with Cox's proportional hazards regression test in primary pTa and pT1 urothelial bladder carcinomas

Prognostic factors	Hazard rate	95% CI	*P*
Stage (pT1 *vs* pTa)	2.51	1.09–5.79	0.031
Size (>3 *vs* <1 cm)	3.82	1.31–11.14	0.0143
Morphology (sessile *vs* pedunculate)	3.53	1.55–8.04	0.0026
KAI1 expression (decreased *vs* positive)	3.92	1.70–9.01	0.0013

CI, confidence interval.

Table 4 Association between *KAI1* expression and recurrence in subgroup according to each clinicopathologic factor and intravesical treatment in primary pTa and pT1 urothelial bladder carcinomas

Prognostic factors	Expression of *KAI1* (–/+)		χ^2 test	
	Recurrence	Non-recurrence	RR (95% CI)	*P*
Histology				
Grade 1	5/4	2/12	7.5 (1.0–55.0)	0.036
Grade 2	16/6	7/27	10.3 (2.9–36.0)	0.0001
Grade 3	2/2	1/3	3.0 (0.2–59.9)	0.47
Pathology				
Stage pTa	6/2	4/21	15.8 (2.3–107.9)	0.0016
Stage pT1	17/10	6/21	6.0 (1.8–19.7)	0.0025
Morphology				
Papillary	20/10	7/38	10.9 (3.6–32.9)	<0.0001
Non-Papillary	3/2	3/4	2.0 (0.2–20.6)	0.56
Pedunculated	11/7	9/33	5.8 (1.7–19.1)	0.0028
Sessile	12/5	1/9	21.6 (2.1–218.6)	0.0023
Size (cm)				
<1	3/3	3/12	4.0 (0.5–30.8)	0.17
1–3	10/5	5/29	11.6 (2.8–48.6)	0.0003
>3	6/4	0/2	–	0.12
Unidentified	4/0	1/0	–	–
No. tumors				
1	11/5	6/24	8.8 (2.2–35.2)	0.001
2–4	8/5	3/9	4.8 (0.9–26.8)	0.07
>5	1/2	0/7	–	0.11
Unidentified	3/0	1/2	–	0.08
Intravesical				
MAC	18/10	9/40	8.0 (2.8–23.1)	<0.0001
BCG	2/1	1/1	2.0 (0.1–78.3)	0.71
Non-treatment	3/1	0/1	–	0.17

BCG, Bacillus Calmette–Guérin; MAC, mitomycin C, adriamycin and cytosine arabinoside; (–/+), decreased/positive expression of *KAI1* protein; RR, relative risk; CI, confidence interval.

higher chance of recurrence among those considered phenotypically at a low risk of recurrence.

Discussion

The *KAI1* gene encodes a protein consisting of 267 amino acids and belonging to a structurally distinct family of leukocyte surface glycoproteins, being a member of the transmembrane 4 superfamily (TM4SF). The *KAI1* gene was isolated from human chromosome 11p11.2 and was shown to suppress metastasis when introduced into rat AT6.1 prostate cancer cells.[16] Decreased expression of this gene may be involved in the malignant progression of prostate and other cancers.[16] Downregulation of the *KAI1* gene during progression of cancer does not commonly involve either mutation or allelic loss of the *KAI1* gene[17] and is not associated with methylation of the promoter or *p53* regulation.[34] Although the mechanisms of action of the

KAI1 protein have not been fully elucidated, some clues are emerging and it seems that, like cell surface glycoproteins, the *KAI1* protein may play an important role in signal transduction.[35]

In the present study, *KAI1* expression was examined using immunohistochemistry.[29,36] Because in the present study patients in both the recurrence and non-recurrence groups had the same type of urothelial carcinoma (transitional cell) and treatment was consistent with the stage of the disease, we were able to analyze the data comparatively. Because *KAI1* is a member of the TM4SF, positive staining for the protein should be observed essentially at the cell membrane. However, in formalin-fixed, paraffin-embedded samples, the modification of which appears to be substantially altered in the presence of the human T-cell leukemia virus type 1 (HTLV-1) genome, immunoreactivity for *KAI1* that could have prognostic value is observed, exhibiting specific cytoplasmic localization of the protein in the cells.[37] In the normal papilla of Vater, esophageal tissue or primary tumor,[18,38] *KAI1* immunoreactivity was also demonstrated in the cytoplasm and at the cell membranes. In the present study, we used a rabbit polyclonal antibody, with epitope mapping at the C-terminus of metastasis suppressor protein (*KAI1*) of human origin, and found that *KAI1* was expressed abundantly in normal urothelial tissue, but was downregulated in pTa and pT1 urothelial bladder carcinoma and further downregulated in muscle-invasive bladder cancers (stage pT2–4). Staining of carcinoma cells was weaker than of normal epithelial cells. We considered decreased *KAI1* protein expression to occur when a non-uniform staining pattern was observed and the proportion of stained cells was less than 50% within the tumor tissue. The non-uniform staining pattern correlates with loss of *KAI1* expression.[25,39] In advanced bladder carcinoma, an abnormal or heterogeneous immunostaining pattern was defined as negative staining and was significantly related to tumor stage and grade.[19] In the present study, these poorest staining patterns were exhibited most at the tumor surface, second at fragments or heterogeneously in fragments and at the base of the tumors. These different staining patterns were also seen at the top more than at base of large tumors, suggesting that cell desquamation maybe more pronounced in pTa and pT1 urothelial bladder carcinoma. Although the malignant activity of pTa and pT1 urothelial bladder carcinoma was lower than the muscle-invasive bladder cancers, decreased expression of *KAI1* also showed a tendency to relate to clinicopathological factors, including grade and stage, and was significantly related to tumor size. This level of *KAI1* expression has been described previously.[40] Our results suggest that this evaluation is a reasonable approach for examining the expression of the *KAI1* metastasis suppressor gene in pTa and pT1 urothelial bladder carcinoma.

Identification of gene expression patterns in superficial and invasive bladder cancer gives us a better understanding of those genes related to encoding proteins involved in cell proliferation, oncogenes and growth factors, cell adhesion, immunology, transcription, proteinases and ribosomes.[41] Recurrence of pTa and pT1 urothelial bladder carcinoma is a complex process in which many genes and steps participate and is related to invasiveness, spread, transplant, adhesion and the proliferation ability of neoplastic cells. The function of the *KAI1* gene is closely correlated to the invasive and reimplantation characteristics of tumor cells in the bladder wall and/or the spreading of tumor cells via expansion within the urothelium, which was thought to be the main cause of the recurrence of pTa and pT1 urothelial bladder carcinoma.[12–15] Univariate and multivariate analysis of results of the present study demonstrated that decreased *KAI1* expression is significantly related to the recurrence of tumors and that it is also an independent prognostic factor of recurrence in pTa and pT1 urothelial bladder carcinoma.

Recurrent tumors have a similar biological potential as the original tumor and behave in a similar manner.[14] To prevent tumor recurrence, it is important to recognize various biological factors and clinicopathological characteristics in each individual case. Previously, we examined the correlation between the tumor repressor gene *p53*, *Ki-67* antigen, *c-erbB-2* oncoprotein and tumor recurrence in pTa and pT1 urothelial bladder carcinoma. When all these factors became negative, the tumor recurrence rate was significantly low, combinational analysis of two factors resulting in a larger significance than analysis of a single factor, but there was no significant correlation between decreased expression of *KAI1* protein and each of these factors. In the present study, subgroup analysis according to each clinicopathological factor showed that patients with decreased *KAI1* expression have a high relative risk of recurrence, even for patients with a low grade, stage pTa, papillae, tumor diameter 1–3 cm and single tumor, which are supposed to indicate a lower recurrence rate. Intravesical treatment, especially BCG instillation, is able to prevent the recurrence of some tumors, but when decreased expression of *KAI1* protein is detected, the follow-up regimen should be evaluated. Although the clinical material available for subgroup analysis is small, tumors were phenotypically similar and the treatment consistent with the characteristics of the tumors, which makes the results more relevant for testing new markers. Thus, analysis of the *KAI1* protein can be useful for medical

practice, because decreased *KAI1* expression occurs during the progression of cancer, when the biological behavior of the tumor changes from low risk to high risk, permitting the identification of those patients in whom the cancer is likely to recur.

In conclusion, decreased expression of *KAI1* protein was related to stage and morphology of the tumor stem and was significantly correlated with tumor size. Decreased *KAI1* expression was associated with the degree of invasiveness and progression of cancer and was an independent prognostic factor for tumor recurrence in primary pTa and pT1 urothelial bladder carcinoma. Additional larger studies are needed to corroborate the validity of these observations and the role of this metastasis suppress gene in the evaluation of primary pTa and pT1 urothelial bladder carcinoma.

Acknowledgments

We thank Professor Taizou Shiraishi (Department of Pathology, Faculty of Medicine, Mie University) for advice on histopathological diagnoses and immunostaining.

References

1 de Vere White RW, Stapp E. Predicting prognosis in patients with superficial bladder cancer. *Oncology* 1998; **12**: 1717–23.
2 Herr HW. Natural history of superficial bladder tumors: 10- to 20-year follow-up of treated patients. *World J. Urol.* 1997; **15**: 84–8.
3 Okajima E, Hirao K. Anti-cancer drug intravesical treatment to superficial bladder tumor. In: Yoshida O (ed.). *Developing with New Bladder Tumor Treatment*. Kanehara, Tokyo, 1993; 105–111 (in Japanese).
4 Takashi M, Murase T, Mitsuya H *et al.* Statistical analysis of recurrence factors in superficial bladder cancer: Estimation with Cox's proportional hazards model. *Jpn. J. Urol.* 1987; **78**: 39–49 (in Japanese).
5 Kobashi K. Statistical analysis of recurrent factors in superficial bladder cancer: Natural history of superficial bladder cancer. *Jpn. J. Urol.* 1993; **84**: 1188–96 (in Japanese).
6 Allard P, Bernard P, Frader Y, Tetu B. The early clinical course of primary Ta and T1 bladder cancer: A proposed prognostic index. *Br. J. Urol.* 1998; **81**: 692–8.
7 Brausi M, Collette L, Kurth K *et al.* Variability in the recurrence rate at first follow-up cystoscopy after TUR in stage Ta T1 transitional cell carcinoma of the bladder: A combined analysis of seven EORTC studies. *Eur. Urol.* 2002; **41**: 523–31.
8 Millan-Rodriguez F, Chechile-Toniolo G, Salvador-Bayarri J, Palou J, Vicente-Rodriguez J. Multivariate analysis of the prognostic factors of primary superficial bladder cancer. *J. Urol.* 2000; **163**: 73–8.
9 Simoneau M, LaRue H, Aboulkassim TO, Meyer F, Moore L, Fradet Y. Chromosome 9 deletions and recurrence of superficial bladder cancer: Identification of four regions of prognostic interest. *Oncogene* 2000; **19**: 6317–23.
10 Krause FS, Feil G, Bichler KH. Immunohistochemical examinations (*Ki67, p53, nm23*) and DNA cytophotometry in bladder cancer. *Anticancer Res.* 2000; **20**: 5023–8.
11 Oosterhuis JW, Schapers RF, Janssen-Heijnen ML, Smeets AW, Pauwels RP. *MIB-1* as a proliferative marker in transitional cell carcinoma of the bladder. *Cancer* 2000; **88**: 2598–605.
12 Simon R, Eltze E, Schafer KL *et al.* Cytogenetic analysis of multifocal bladder cancer supports a monoclonal origin and intraepithelial spread of tumor cells. *Cancer Res.* 2001; **61**: 355–62.
13 van Tilborg AA, de Vries A, de Bont M, Groenfeld LE, van der Kwast TH, Zwarthoff EC. Molecular evolution of multiple recurrent cancers of the bladder. *Hum. Mol. Genet.* 2000; **9**: 2973–80.
14 Dalbagni G, Ren ZP, Herr H, Cordon-Cardo C, Reuter V. Genetic alterations in *TP53* in recurrent urothelial cancer. A longitudinal study. *Clin. Cancer Res.* 2001; **7**: 2797–801.
15 Hartmann A, Rosner U, Schlake G *et al.* Clonality and genetic divergence in multifocal low-grade superficial urothelial carcinoma as determined by chromosome 9 and p53 deletion analysis. *Lab. Invest.* 2000; **80**: 709–18.
16 Dong JT, Lamb PW, Rinker-Schaeffer CW *et al. KAI1*, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2. *Science* 1995; **268**: 884–6.
17 Dong JT, Suzuki H, Pin SS *et al.* Down-regulation of the *KAI1* metastasis suppressor gene during the progression of human prostatic cancer infrequently involves gene mutation or allelic loss. *Cancer Res.* 1996; **56**: 4387–90.
18 Friess H, Guo XZ, Tempia-Caliera AA *et al.* Differential expression of metastasis-associated genes in papilla of Vater and pancreatic cancer correlates with disease stage. *J. Clin. Oncol.* 2001; **19**: 2422–32.
19 Ow K, Delprado W, Fisher R *et al.* Relationship between expression of the *KAI1* metastasis suppressor and other markers of advanced bladder cancer. *J. Pathol.* 2000; **191**: 39–47.
20 Huang CI, Kohno N, Ogawa E, Adachi M, Taki T, Miyake M. Correlation of reduction in *MRP-1/CD9* and *KAI1/CD82* expression with recurrences in breast cancer patients. *Am. J. Pathol.* 1998; **153**: 973–83.
21 Yang X, Wei LL, Tang C, Slack R, Mueller S, Lippman ME. Overexpression of *KAI1* suppresses *in vitro* invasiveness and *in vivo* metastasis in breast cancer cells. *Cancer Res.* 2001; **61**: 5284–8.
22 Takaoka A, Hinoda Y, Satoh S *et al.* Suppression of invasive properties of colon cancer cells by a metastasis suppressor *KAI1* gene. *Oncogene* 1998; **16**: 1443–53.
23 Takaoka A, Hinoda Y, Sato S *et al.* Reduced invasive and metastatic potentials of kai1-transfected melanoma cells. *Jpn. J. Cancer Res.* 1998; **89**: 397–404.

24 Jackson P, Kingsley EA, Russell PJ. Inverse correlation between *KAI1 mRNA* levels and invasive behaviour in bladder cancer cell lines. *Cancer Lett.* 2000; **156**: 9–17.
25 Uchida S, Shimada Y, Watanabe G *et al.* Motility-related protein (*MRP-1/CD9*) and KAI1/CD82 expression inversely correlate with lymph node metastasis in oesophageal squamous cell carcinoma. *Br. J. Cancer* 1999; **79**: 1168–73.
26 Lombardi DP, Geradts J, Foley JF, Chiao C, Lamb PW, Barrett JC. Loss of *KAI1* expression in the progression of colorectal cancer. *Cancer Res.* 1999; **59**: 5724–31.
27 Liu FS, Chen JT, Dong JT *et al.* KAI1 metastasis suppressor gene is frequently down-regulated in cervical carcinoma. *Am. J. Pathol.* 2001; **159**: 1629–34.
28 Liu FS, Dong JT, Chen JT, Hsieh YT, Ho ES, Hung MJ. Frequent down-regulation and lack of mutation of the *KAI1* metastasis suppressor gene in epithelial ovarian carcinoma. *Gynecol. Oncol.* 2000; **78**: 10–15.
29 Higashiyama M, Kodama K, Yokouchi H *et al. KAI1/CD82* expression in nonsmall cell lung carcinoma is a novel, favourable prognostic factor. *Cancer* 1998; **83**: 466–74.
30 Japanese Urological Association and the Japanese Society of Pathology. *General Rule for Clinical and Pathological Studies on Bladder Cancer*. Kanehara, Tokyo, 2001.
31 Sobin LH, Wittekind C. *TNM Classification of Malignant Tumors*. Wiley-Liss, New York, 1997.
32 Mostofi FK, Davis CJ, Sesterhenn IA. *Histological typing of urinary bladder tumors*. In: *World Health Organization International Histological Classification of Tumor*, 2nd edn. Springer, New York, 1999.
33 Pfister C, Moore L, Allard P *et al.* Predictive value of cell cycle markers *p53*, *MDM2*, *p21*, and *Ki-67* in superficial bladder tumor recurrence. *Clin. Cancer Res.* 1999; **5**: 4079–84.
34 Uzawa K, Ono K, Suzuki H *et al.* High prevalence of decreased expression of *KAI1* metastasis suppressor in human oral carcinogenesis. *Clin. Cancer Res.* 2000; **28**: 828–35.
35 Wright MD, Tomlinson MG. The ins and outs of the transmembrane 4 superfamily. *Immunol. Today* 1994; **15**: 588–94.
36 Geradts J, Maynard R, Birrer MJ *et al.* Frequent loss of *KAI1* expression in squamous and lymphoid neoplasms: An immunohistochemical study of archival tissues. *Am. J. Pathol.* 1999; **154**: 1665–71.
37 Fukudome K, Furuse M, Imai T *et al.* Identification of membrane antigen *C33* recognized by monoclonal antibodies inhibitory to human T-cell leukemia virus type I (HTLV-1)-induced syncytium formation: Altered glycosylation of *C33* antigen in HTLV-1-positive T cells. *J. Virol.* 1992; **66**: 1394–401.
38 Miyazaki T, Kato H, Shitara Y *et al.* Mutation and expression of the metastasis suppressor gene *KAI1* in esophageal squamous cell carcinoma. *Cancer* 2000; **89**: 955–62.
39 Shinohara T, Miki T, Nishimura N *et al.* Nuclear factor-kappaB-dependent expression of metastasis suppressor *KAI1/CD82* gene in lung cancer cell lines expressing mutant p53. *Cancer Res.* 2001; **61**: 673–8.
40 Yu Y, Yang JL, Markovic B *et al.* Loss of *KAI1* messenger RNA expression in both high-grade and invasive human bladder cancers. *Clin. Cancer Res.* 1997; **3**: 1045–9.
41 Thykjaer T, Workman C, Kruhoffer M *et al.* Identification of gene expression patterns in superficial and invasive human bladder cancer. *Cancer Res.* 2001; **61**: 2492–9.

0022-5347/04/1724-1480/0
THE JOURNAL OF UROLOGY®

Vol. 172, 1480–1484, October 2004
Printed in U.S.A.
DOI: 10.1097/01.ju.0000137749.99255.3e

IN SITU GELATINOLYTIC ACTIVITY CORRELATES WITH TUMOR PROGRESSION AND PROGNOSIS IN PATIENTS WITH BLADDER CANCER

KOJI KAWAMURA, NAOTO KAMIYA, TAKAHITO SUYAMA, MASAKI SHIMBO, NOBUYUKI OOSUMI, HIROYOSHI SUZUKI, TAKESHI UEDA, TOYOFUSA TOBE, TATSUO IGARASHI,* HARUO ITO AND HIROSHI ISHIKURA

From the Departments of Urology (KK, NK, TS, MS, NO, HS, TU, TT, TI, HI) and Molecular Pathology (TS, HI), Graduate School of Medicine, Chiba University, Chiba, Japan

ABSTRACT

Purpose: Degradation of the extracellular matrix by malignant tumor cells has an essential role in the process of tumor invasion and metastasis. The 2 gelatinolytic matrix metalloproteinases (MMPs) MMP-2 and MMP-9 are believed to be key enzymes in this process. We investigated the possible relationship between *in situ* gelatinolytic activity of MMPs and clinicopathological factors in patients with bladder cancer to clarify whether these proteins would be critical for tumor advancement in this disease.

Materials and Methods: We evaluated the intensity of gelatinolytic activity in 25 bladder cancer tissues by film *in situ* zymography (FIZ). To clarify the MMP(s) responsible for gelatinolytic activity in bladder cancer tissues we examined MMP-2 and MMP-9 expression in bladder tissues by gelatin zymography. MMP expression was also confirmed by reverse transcriptase-polymerase chain reaction and Western blotting. We then investigated the association between MMP expression detected by gelatin zymography and the intensity of gelatinolytic activity determined by FIZ.

Results: FIZ demonstrated that all tumor tissues had *in situ* gelatinolytic activities. There was a statistically significant correlation between the intensity of gelatinolytic activity, and tumor grade, stage, vessel invasion and cause specific survival ($p < 0.05$). Stronger *in situ* gelatinolytic patterns were documented in cases with higher pro and active MMP-2 expression.

Conclusions: FIZ enables the direct assessment of *in situ* gelatinolytic activity in bladder cancer tissues. The intensity of activity appears to affect the biology of carcinoma tissues. Our results indicate a major role for MMP-2 in *in situ* gelatinolysis in bladder cancer.

KEY WORDS: bladder, bladder neoplasms, matrix metalloproteinases, gelatinases

Bladder cancer is the second common malignant disease in the urogenital organs and its incidence is still increasing. There are several therapeutic options for bladder cancer, such as transurethral resection, radical cystectomy, instillation of some chemoagents or bacillus Calmette-Guerin, chemotherapy and combinations of these therapeutic modalities. Tumor stage is a strong prognostic factor and it provides information when selecting therapeutic modalities. However, there seems to be some discrepancy between tumor stage and malignant potential in this disease. Therefore, the establishment of adequate biological parameter is mandatory to add useful information on stage.

Tumor cell invasion and metastasis biologically depend on the proteolytic destruction of surrounding matrix components. Matrix metalloproteinases (MMPs) are a family of enzymes that are capable of degrading the basement membrane and extracellular matrix. To date about 20 MMPs have been recognized. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are believed to have an important role in destruction of the basement membrane. These enzymes are considered to contribute to tumor invasion and metastasis. There have been several reports regarding the prognostic significance of increased MMP expression in cancers in several human organs.[1–12]

Accepted for publication April 23, 2004.
* Correspondence: Department of Urology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan (telephone: +81-43-226-2134; FAX: +81-43-226-2136; e-mail: igarashi@faculty.chiba-u.jp).

Film *in situ* zymography (FIZ)[13–17] can directly show *in situ* gelatinolytic activity in tissue sections. We have already reported that gelatinolytic activity on FIZ correlates with the size, grade and vessel invasion of renal cell carcinomas, and MMP-2 activity correlates with gelatinolytic activity on FIZ.[14] In this study we investigated the possible relationship between FIZ findings and various clinicopathological factors in patients with bladder carcinoma. We also investigated the correlation between MMP expression detected by gelatin zymography (GZG) and the intensity of *in situ* gelatinolytic activity.

MATERIALS AND METHODS

Patients and tissue specimens. A total of 25 patients with bladder carcinoma treated at the department of urology at our institution between 1995 and 2002 were included in the current study. Mean age was 66.7 years (range 41 to 88). The study group comprised 24 men and 1 woman. All samples were obtained with informed consent. All patients had undergone total cystectomy or transurethral bladder tumor resection. Tumor samples were snap frozen in liquid nitrogen, embedded in Tissue-Tek OCT compound (Sakura Finetechnical Co., Ltd., Tokyo, Japan), and stored at −80C. All hematoxylin and eosin stained slides from each case were reviewed and all tumors were histologically classified as transitional cell carcinoma (TCC). All Victoria blue stained

slides were also reviewed to confirm vessel invasion (positive or negative).

FIZ. Frozen sections were cut 4 μm thick using a cryotome and mounted on polyethylenetelephthalate film coated with 7% gelatin (FIZ-GN) (Fuji Photo Film Co., Ltd., Tokyo, Japan). As a control, these sections were mounted on polyethylenetelephthalate film coated with 7% gelatin including MMP inhibitor (FIZ-GI, 1,10-phenanthroline, Sigma Chemical Co., St. Louis, Missouri). At the same time hematoxylin and eosin staining was performed. They were incubated in a moist chamber at 37C for 12 hours and the films were stained with 0.5% Amido Black 10B (Wako Co., Ltd., Osaka, Japan) for 15 minutes. The films were then destained for 15 minutes with a solution containing 70% methanol and 10% acetic acid. Gelatinolytic activity was determined as a clearly visible area after unstaining by Amido Black 10B. The intensity of *in situ* gelatinolysis was classified into 4 patterns according to visualized degradation on FIZ-GN films, namely pattern A—no gelatinolytic activity, pattern B—weak activity with gelatin films scarcely dissolved, pattern C—focal complete degradation and pattern D—diffuse complete degradation (fig. 1).

GZG. The GZG method was described in the previous report. Enzymatic activities of MMP-2, proMMP-2, MMP-9 and proMMP-9 were determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis gelatin zymography. Normal and malignant frozen bladder tissues were homogenized. Supernatants were extracted and adjusted to a protein concentration of 1 μg/ml. We used supernatants of HT1080 (fibrosarcoma) cells as a positive control and an MMP sample (Yagai Laboratories, Yamagata, Japan) as a marker. All samples were subjected to nonreduced SDS-polyacrylamide gel electrophoresis using 10% polyacrylamide gels containing 7.5% gelatin. Subsequently SDS was removed by soaking gels in 2.5% Triton X-100 solution for 1 hour at room temperature. The gels were re-incubated at 37C for 24 hours in reaction buffer containing 50 mmol/ml tris-HCl (pH 7.6), 100 mmol/l NaCl, 10 mmol/ml $CaCl_2$, 0.05% Brij-35 and 0.02% NaN_3, stained with Coomassie brilliant blue and destained. Gels were then dried for use with Gel Dry Solution (Tefco, Tokyo, Japan).

The gelatinolytic activities of proMMP-2, MMP-2, proMMP-9 and MMP-9, as detected by GZG were quantified using a densitometer (Model GS-700 Imaging Densitometer, BioRad Laboratories, Hercules, California). The ratio of band density of each sample to that of the standard marker was determined.

RNA extraction and reverse transcriptase (RT)-polymerase chain reaction (PCR). Total RNA was isolated from 2 tissue samples of each FIZ pattern group using Trizol reagent (Invitrogen, Carlsbad, California). RNA (1 μg) was reverse transcribed to cDNA in a final volume of 20 μl containing oligo deoxythymidine primers, 25 mmol/l $MgCl_2$, tris-HCl, 10 mmol/l deoxynucleoside triphosphate mix and SuperScript II RT (Invitrogen). PCR primers for human MMP-2 were forward 5′-AAG GCC AAG TGG TCC GTG TGA A-3′ and reverse 5′-AAC AGT GGA CAT GGC GGT CTC AG-3′, amplifying a 371 bp fragment, and for MMP-9 they were forward 5′-GGC GCT CAT GTA CCC TAT GT-3′ and reverse 5′-TCA AAG ACC GAG TCC AGC TT-3′, amplifying a 468 bp fragment.

PCR was done in a 20 μl volume containing first strand cDNA, forward primer, reverse primer, buffer, $MgCl_2$ and Taq polymerase (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom). For amplification 35 cycles of a 3-step temperature profile was performed, including denaturation at 94C for 60 seconds, annealing at 54C for 30 seconds and extension for 60 seconds with a final extension for 5 minutes at 72C. As a control, the internal standard β-actin was used according to manufacturer instructions (Invitrogen). A 6 μl volume of PCR mixture was electrophoresed on 1.5% agarose gel and stained with ethidium bromide.

Western blot analysis. Two tissue samples from each FIZ pattern group were used to prepare protein extracts. After extraction tumorous and normal samples were adjusted to include 100 μg protein, electrophoresed in 10% SDS-polyacrylamide gel and transferred to polyvinylidine difluoride (PVDF) membranes by electroblotting. PVDF membranes were incubated with primary antibodies at 1:1,000 dilution overnight at 4C. Two primary antibodies against human MMP-2 and MMP-9 (Sigma Chemical Co.) were used. After extensive washing of PVDF membranes antibodies were incubated with peroxidase conjugated horseradish antirabbit IgG (Amersham Pharmacia Biotech) at 1:1,000 dilution for 1 hour at 4C. MMP bands were detected using echoluminescence Western blotting detection systems (Amersham Pharmacia Biotech).

Statistical analysis. Statistical significance was calculated using the Mann-Whitney U-test, Kruskal-Wallis test, 1-way ANOVA or simple regression. Survival curves were computed using the Kaplan-Meier method with the log rank test with $p < 0.05$ considered significant.

RESULTS

Gelatinase activity was observed in all cancerous tissues by FIZ. Gelatinolytic activity was decreased on FIZ-GI films in all tissues compared to FIZ-GN films (fig. 2), indicating that MMPs have a major role in the *in situ* gelatinolytic activity of tumorous tissue in bladder cancer. Table 1 shows the correlation between gelatinolytic activity patterns and clinicopathological factors. There was a statistically significant correlation between *in situ* gelatinolytic activity patterns and prognostic factors such as tumor grade, stage and vessel invasion ($p < 0.05$, table 1). However, no relationship was observed between the patterns and intraluminal recurrence ($p \geqq 0.05$).

We divided *in situ* gelatinolytic activity patterns into 2 groups, that is nondiffuse—patterns A to C and diffuse—pattern D. When the patterns were grouped in this manner, cause specific survival was significant in the diffuse activity group ($p < 0.05$, fig. 3).

The pro and active forms of MMP-2, and pro-MMP-9 were detected in all tissues by GZG. However, the active form of MMP-9 was not detected in all bladder samples examined (fig. 4). The intensity of MMP-2 and pro-MMP-2 expression on GZG clearly corresponded to that of the *in situ* gelatinolytic activity pattern. On the contrary, proMMP-9 or the MMP-2-to-proMMP-2 ratio did not correlate to *in situ* gelatinolytic activity patterns (table 2).

RT-PCR and Western blot analysis confirmed the expression of MMP-2 and MMP-9 mRNA and protein in all tumorous and normal bladder tissues examined (figs. 5 and 6).

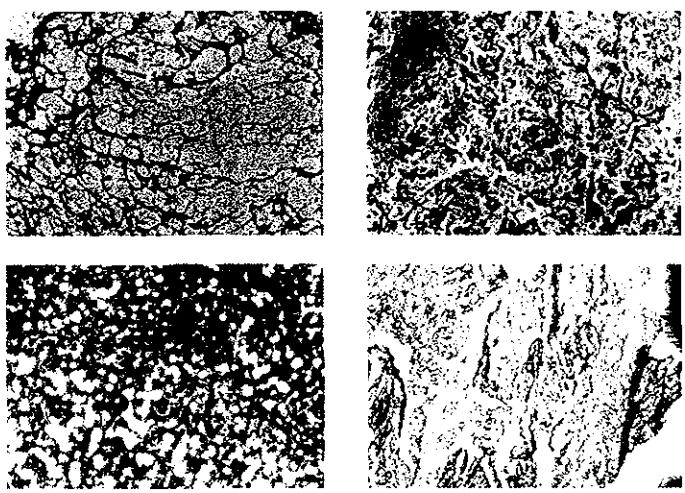

FIG. 1. Intensity patterns of *in situ* gelatinolytic activity exerted by bladder cancer tissues. *a*, pattern A—no activity. *b*, pattern B—weak activity. *c*, pattern C—focal complete gelatinolysis. *d*, pattern D—diffuse complete gelatinolysis. Reduced from ×100.

FIG. 2. *In situ* gelatinolytic activity in bladder cancer tissue. *a*, frozen section stained with hematoxylin and eosin. *b*, *in situ* gelatinolytic activity. *c*, suppression of *in situ* gelatinolytic activity by MMP inhibitor 1,10-phenanthroline. Most activity in cancer tissue was suppressed. Reduced from ×40.

TABLE 1. *FIZ patterns and clinicopathological factors in bladder cancer*

	Total No. Pts	No. FIZ Pattern				p Value
		A	B	C	D	
No. pts		0	11	5	9	
Tumor stage:						
pTa	5	–	4	1	–	0.0284
pT1	10	–	6	2	2	
pT2a	4	–	2	2	–	
pT2b	1	–	–	–	1	
pT3	3	–	–	–	3	
pT4	2	–	–	–	2	
Local recurrence:						
Neg	15	–	6	6	4	0.563
Pos	10	–	6	8	8	
Grade:						
G1	3	–	2	–	1	0.029
G2	13	–	8	3	2	
G3	9	–	1	2	6	
Vessel invasion:						
Neg	14	–	9	3	2	0.006
Pos	11	–	2	2	7	

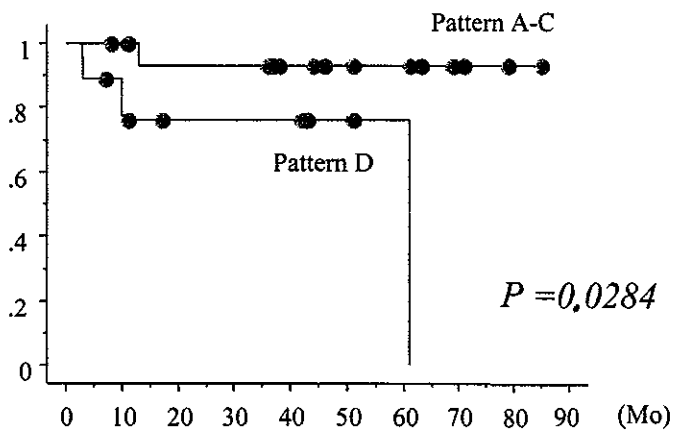

FIG. 3. Kaplan-Meier cause specific survival curves according to FIZ pattern. Diffuse groups (pattern D) showed worse cause specific survival than nondiffuse groups (patterns A to C).

DISCUSSION

Bladder cancer can be dichotomized according to distinct biological activity and prognosis as low grade noninvasive tumors and high grade invasive tumors. Briefly, bladder cancer consists of noninvasive tumors (less than T1) in 75% to 85% of patients and invasive (T2 or greater) or metastatic tumors in 15% to 25%. More than 70% of patients with noninvasive cancer have 1 or more recurrences after initial treatment. Primary therapy is based on clinical staging and morphological assessment. However, tumors may behave differently despite morphological similarity. It is of great importance to identify patients who are likely to have recurrent or progressive disease. In addition to conventional prognostic factors such as stage and grade, FIZ may be a new prognostic marker with which to identify patients at risk for future progression.

MMP-2 and MMP-9 have the ability to degrade type IV collagen and they are believed to have an important role in destruction of the basement membrane. MMP activity is regulated by 2 mechanisms. 1) The secreted latent proenzyme forms of MMPs must undergo proteolytic activasion. 2) Ubiquitous tissue inhibitor of metalloproteinase (TIMP) can interfere with MMP proteolytic activation and enzymatic activity.

Davies et al measured levels of MMP-2 and MMP-9 in 42 TCC tissues and 7 normal bladder tissues using quantitative GZG.[10] The levels of MMP-9 and active MMP-2 correlated with higher tumor grade and invasion, suggesting the prognostic value of MMPs for carcinoma of the bladder. Kanayama et al investigated the expression of MMP-2, TIMP-2 and MT1-MMP by RT-PCR in 41 TCC tissues.[11] They found that MMP-2 and TIMP-2 expression was significantly higher in muscle invasive than in superficial tumors and high MMP-2, TIMP-2 and MT1-MMP expression was significantly associated with decreased survival. In addition, Gerhards et al used GZG to measure MMP-2 and MMP-9 excretion in the urine of patients with bladder cancer to evaluate their diagnostic clinical validity.[12] They found that urinary excretion of MMP-2 and MMP-9 was associated with a high stage and grade of bladder cancer.

These studies resulted in the detection of MMP/TIMP proteins or mRNA in bladder carcinoma tissues. However, GZG and RT-PCR cannot directly detect *in situ* gelatinolytic activity. In contrast, FIZ enables us to detect *in situ* gelatinolytic activity. To our knowledge the current study is the first to examine *in situ* gelatinolytic activity in relation to clinicopathologic factors in bladder carcinoma.

Our results show that MMP-2 activity but not MMP-9 activity correlates with the gelatinolytic activity of bladder

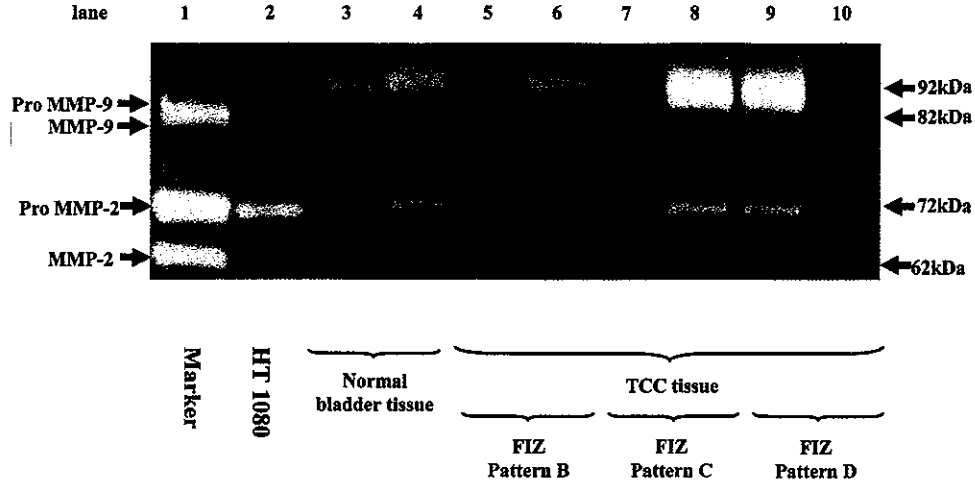

FIG. 4. MMP profiles of representative bladder cancer cases with different *in situ* gelatinolysis patterns on GMZ. ProMMP-2, MMP-2 and proMMP-9 were detected in all cases, while MMP-9 expression was not seen.

TABLE 2. *FIZ patterns, and expression of pro and activated MMPs forms by GZG*

	Mean FIZ Pattern ± SE			p Value
	B	C	D	
No. pts	11	5	9	
proMMP-9	0.90 ± 0.20	0.97 ± 0.09	1.12 ± 0.46	0.40
proMMP-2	0.24 ± 0.12	0.31 ± 0.15	0.74 ± 0.23	0.0004
MMP-2	0.15 ± 0.07	0.20 ± 0.08	0.45 ± 0.16	0.0008
MMP-2/proMMP-2	0.65 ± 0.14	0.67 ± 0.07	0.62 ± 0.19	0.73

MMPs amounts are expressed as a relative value, as quantified by a densitometer.

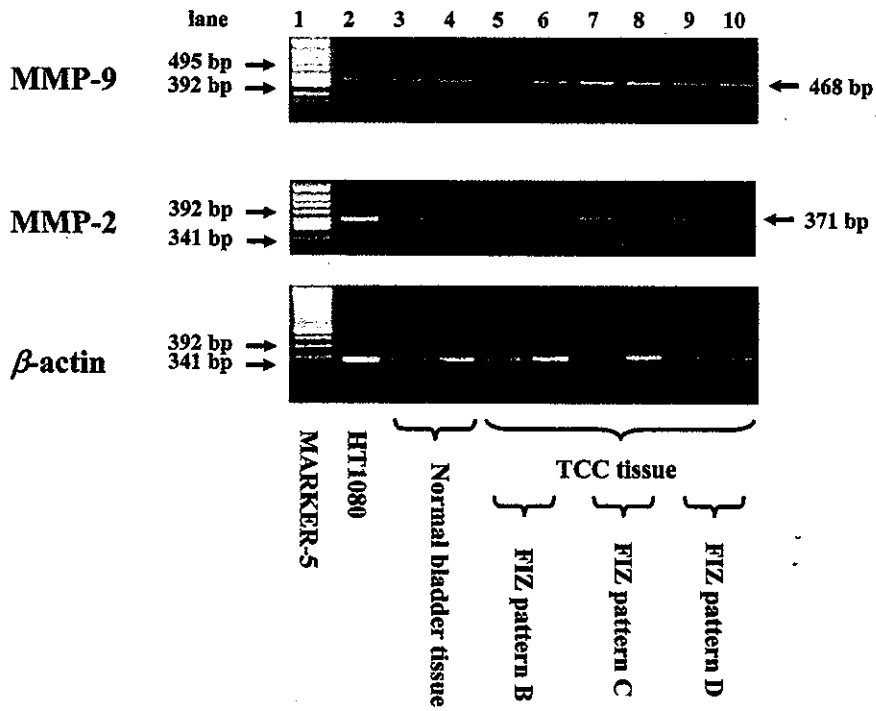

FIG. 5. MMP mRNA production by bladder cancer tissues. Virtually all tissues expressed MMP-2 and MMP-9 mRNA

carcinoma *in vivo*. Gelatinolytic activity correlates with tumor grade, stage, vessel invasion and cause specific survival. These results suggest that MMP-2 activity is an important factor in the *in vivo* biology of bladder carcinomas.

Using FIZ, Ikeda et al examined gelatinolytic activity in implanted tumor tissues, finding that activated MMP-2 but not pro-MMP-2 shows gelatinolytic activity in FIZ.[15] They also noted that gelatinolytic activity in their experimental system was derived primarily from MMPs. They suggested that FIZ can detect net MMP activity in tumor tissues. Our previous study also demonstrated that activated MMP-2 shows gelatinolytic activity in FIZ and gelatinolytic activity examined by FIZ correlates with tumor size, grade and vessel invasion of renal cell carcinomas.[14,17]

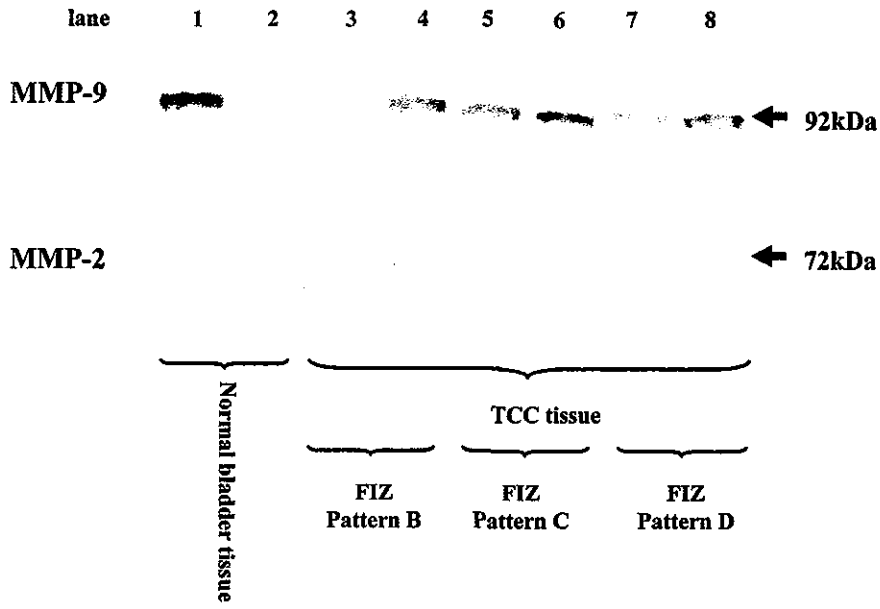

FIG. 6. Production of 72 kDa MMP-2 and 92 kDa MMP-9 by bladder cancer tissues. Virtually all tissues expressed antigens

CONCLUSIONS

Our results indicate fundamental biochemical differences between low and high grade bladder tumors. Different FIZ patterns may explain differences in invasive and metastatic behavior among tumors. FIZ may be a useful prognostic indicator in patients with bladder carcinoma and it may be helpful for designing treatment protocols. For example, the clinical point is the timing of radical cystectomy for G3 pT1 bladder carcinomas because delayed cystectomy is directly related to a serious threat to patient survival. FIZ may assist in choosing treatment for G3 pT1 bladder carcinomas.

REFERENCES

1. Shima, I., Sasaguri, Y., Kusukawa, J., Yamana, H., Fujita, H., Kakegawa, T. et al: Production of matrix metalloproteinases-2 and metalloproteinases-3 related to malignant behavior of esophageal carcinoma. A clinicopathologic study. Cancer, **70:** 2747, 1992
2. Sier, C. F., Kubben, F. J., Ganesh, S., Heerding, M. M., Griffioen, G., Hanemaaijer, R. et al: Tissue levels of matrix metalloproteinases MMP-2 and MMP-9 are related to the overall survival of patients with gastric carcinoma. Br J Cancer, **74:** 413, 1996
3. Liabakk, N. B., Talbot, I., Smith, R. A., Wilkinson, K. and Balkwill, F.: Matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9) type IV collagenases in colorectal cancer. Cancer, **56:** 190, 1996
4. Talvensaari-Mattila, A., Paakko, P., Hoyhtya, M., Blanco-Sequeiros, G. and Turpeenniemi-Hujanen, T.: Matrix metalloproteinase-2 immunoreactive protein: a marker of aggressiveness in breast carcinoma. Cancer, **83:** 1153, 1998
5. Bramhall, S. R., Stamp, G. W., Dunn, J., Lemoine, N. R. and Neoptolemos, J. P.: Expression of collagenase (MMP2), stromelysin (MMP3), and tissue inhibitor of the metalloproteinases (TIMP1) in pancreatic and ampullary disease. Br J Cancer, **73:** 972, 1996
6. Stearns, M. and Stearns, M. E.: Evidence for increase activated metalloproteinase 2 (MMP-2a) expression associated with human prostate cancer progression. Oncol Res, **8:** 69, 1996
7. Montironi, R., Lucarini, G., Castaldini, C., Galluzzi, C. M., Biagini, G. and Fabris, G.: Immunohistochemical evaluation of type IV collagenase (72-kd metalloproteinase) in prostatic intraepithelial neoplasia. Anticancer Res, **16:** 2057, 1996
8. Kallakury, B. V., Karikehalli, S., Haholu, A., Sheehan, C. E., Azumi, N. and Ross, J. S.: Increased expression of matrix metalloproteinases 2 and 9 and tissue inhibitors of metalloproteinases 1 and 2 correlate with poor prognostic variables in renal cell carcinoma. Clinic Cancer Res, **7:** 3113, 2001
9. Kugler, A., Hemmerlein, B., Thelen, P., Kallerhoff, M., Radzun, H.-J. and Ringert, R.-H.: Expression of metalloproteinase 2 and 9 and their inhibitors in renal cell carcinoma. J Urol, **160:** 1914, 1998
10. Davies, B., Waxman, J., Wasan, H., Abel, P., Williams, G., Krausz, T. et al: Levels of matrix metalloproteinases in bladder cancer correlate with tumor grade and invasion. Cancer Res, **53:** 5365, 1993
11. Kanayama, H., Yokota, K., Kurokawa, Y., Murakami, Y., Nishitani, M. and Kagawa, S.: Prognostic values of matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-2 expression in bladder cancer. Cancer, **82:** 1359, 1998
12. Gerhards, S., Jung, K., Koenig, F., Daniltchenko, D., Hauptmann, S., Schnorr, D. et al: Excretion of matrix metalloproteinases 2 and 9 in urine is associated with a high stage and grade of bladder carcinoma. Urology, **57:** 675, 2001
13. Furuya, M., Ishikura, H., Nemori, R., Shibata, M., Fujimoti, S. and Yoshiki, T.: Clarification of the active gelatinolytic sites in human ovarian neoplasms using in situ zymography. Hum Pathol, **32:** 163, 2001
14. Zheng, K., Nagai, Y., Kishimoto, T., Yamazawa, K., Tate, S., Nemori, R. et al: A quantitative evaluation of active gelatinolytic sites in uterine endometrioid adenocarcinoma using film in situ zymography: association of stronger gelatinolysis with myometrial invasion. Jpn J Cancer Res, **93:** 516, 2002
15. Ikeda, M., Maekawa, R., Tanaka, H., Matsumoto, M., Takeda, Y., Tamura, Y. et al: Inhibition of gelatinolytic activity in tumor tissues by synthetic matrix metalloproteinase inhibitor: application of film in situ zymography. Clinic Cancer Res, **6:** 3290, 2000
16. Lengyel, E., Schmalfeldt, B., Konik, E., Spathe, K., Harting, K., Fenn, A. et al: Expression of latent matrix metalloproteinase 9 (MMP-9) predicts survival in advanced ovarian cancer. Gynecol Oncol, **82:** 291, 2001
17. Kamiya, N., Kishimoto, T., Suzuki, H., Sekita, N., Nagai, Y., Oosumi, N. et al: Increased in situ gelatinolytic activity in renal cell tumor tissues correlates with tumor size, grade and vessel invasion. Int J Cancer, **106:** 480, 2003
18. Ozdemir, E., Kakehi, Y., Okuno, H. and Yoshida, O.: Role of matrix metalloproteinase-9 in the basement membrane destruction of superficial urothelial carcinomas. J Urol, **161:** 1359, 1999
19. Hara, I., Miyake, H., Hara, S., Arakawa, S. and Kamidono, S.: Significance of matrix metalloproteinases and tissue inhibitors of metalloproteinase expression in the recurrence of superficial transitional cell carcinoma of the bladder. J Urol, **165:** 1769, 2001
20. Papathoma, A. S., Petraki, C., Grigorakis, A., Papakonstantinou, H., Karavana, V., Stefanakis, S. et al: Prognostic significance of matrix metalloproteinases 2 and 9 in bladder cancer. Anticancer Res, **20:** 2009, 2000

Int J Clin Oncol (2004) 9:125–129
DOI 10.1007/s10147-003-0379-8

ORIGINAL ARTICLE

Senji Hoshi · Chikara Ohyama · Kunio Ono
Atsushi Takeda · Shinichi Yamashita · Takashi Yamato
Akihiro Itoh · Makoto Satoh · Seiichi Saito
Yasuhiro Okada · Fumihiko Sohma · Yoichi Arai

Gemcitabine plus carboplatin; and gemcitabine, docetaxel, and carboplatin combined chemotherapy regimens in patients with metastatic urothelial carcinoma previously treated with a platinum-based regimen: preliminary report

Received: August 25, 2003 / Accepted: December 18, 2003

Abstract
Background. The aim of this study was to evaluate the efficacy and safety of two combined chemotherapy regimens in the treatment of previously treated metastatic urothelial carcinoma: gemcitabine plus carboplatin (GC), and gemcitabine, docetaxel, and carboplatin (GDC).
Methods. Sixteen patients with metastatic urothelial cancer, previously treated with a platinum-based regimen, were studied. GC (gemcitabine 750 mg/m^2, on days 1, 8, and 15; carboplatin 200 mg/m^2, on day 2) was administered every 28 days to 15 patients. GDC (gemcitabine 750 mg/m^2, on days 1 and 8; docetaxel 50 mg/m^2, on day 1; carboplatin 200 mg/m^2 on day 1) was administered every 21 days to 9 patients. Eight of the 9 GDC-treated patients had earlier been treated with GC and had become refractory.
Results. With the GC therapy, 7 of the 15 treated patients (47%; 95% confidence interval, 21%–73%) showed an objective response, with 3 achieving a clinical complete response (CR) and 4 a partial response (PR). With the GDC therapy, 6 of the 9 treated patients (67%; 95% confidence interval, 29%–92%) showed an objective response, with 1 achieving CR and 5, PR. Five of the 8 (63%) GC-refractory patients responded to GDC therapy. The median duration of response was 4 months (range, 2–10+ months) on GC therapy, and 3 months (range, 3–5 months) on GDC therapy. Toxicities associated with GC were less than those with GDC.
Conclusion. GC was effective for refractory metastatic urothelial cancer, and GDC was effective for GC-refractory cancer.

Key words Gemcitabine · Docetaxel · Metastatic urothelial cancer

S. Hoshi (✉) · K. Ono
Department of Urology, Yamagata Prefectural Medical Center for Cancer and Life-Style Related Disease, 1800 Aoyagi, Yamagata 990-2292, Japan
Tel. +81-23-685-2626; Fax +81-23-685-2608
e-mail: hoshi@uro.med.tohoku.ac.jp

C. Ohyama
Department of Urology, Akita University School of Medicine, Akita, Japan

A. Takeda · S. Yamashita · T. Yamato · A. Itoh · M. Satoh · S. Saito · Y. Arai
Department of Urology, Tohoku University Graduate School of Medicine, Sendai, Japan

Y. Okada
Department of Urology, Kesennuma City Hospital, Kesennuma, Japan

F. Sohma
Department of Urology, Hachinohe City Hospital, Hachinohe, Japan

Introduction

Gemcitabine, a cell-cycle-specific pyrimidine nucleoside analog, is converted within the cell to triphosphate metabolites. The incorporation of gemcitabine triphosphate into actively replicating DNA and masked-chain termination results in the inhibition of DNA synthesis.[1,2]

Gemcitabine exhibits significant activity in metastatic transitional cell cancer (TCC), with minimal toxicity, but it has little effect on increasing patient survival. Trials of gemcitabine in combination with other active agents have thus been suggested.[3] Paclitaxel was originally a natural product derived from the bark of the North American yew tree, *Taxus brevifola*. Clinical studies using paclitaxel commenced in the mid-1980s. French researchers produced an extract of the European yew, *Taxus baccata*, and modified it with a chemically synthesized side chain. Docetaxel emerged as a result of these efforts and entered clinical trials in 1990.[4] Docetaxel is capable of inducing bcl2 phosphorylation and apoptotic cell death at 100-fold lower concentrations than paclitaxel.[5]

At present, the combination of cisplatin, methotrexate, doxorubicin, and vinblastine (M-VAC)[6] is most widely used for advanced TCC and has shown overall response rates of 40%–72% in phase II studies and 35%–45% in phase III studies, with a median survival of approximately 12 months. These modest results and unsuccessful attempts to increase

Table 1. Characteristics of patients receiving GC and/or GDC chemotherapy

Patient no.	Age (years)	Sex	Disease site	Previous chemotherapy	Response to GC	Response duration (months)	Response to GDC	Response duration (months)	Survival after GC and/or GDC chemotherapy (months)
1	52	M	LN, lung	MEC, M-VAC	PD				3
2	72	M	LN	M-VAC, ITP	CR	5			8
3	68	M	Bone	MEC, ITP	NC				24+
4	56	M	Bone	MEC, M-VAC	PD				8+
5	68	M	LN	MEC, ITP	CR	5			8+
6	71	M	LN	MEC, ITP	CR	10+			11+
7	61	M	LN, Lung, liver	ITP	PD				3
8	46	M	Lung	MEC	PD		PR	3	12+
9	68	M	Lung	MEC	PR	3	CR	3	6
10	73	M	Liver	M-VAC	PR	8	PR	5	15
11	66	M	Lung	MEC	PR	2	PR	3	4+
12	68	M	LN	MEC, ITP	PD		PD		10+
13	51	M	LN, lung, liver	MEC, ITP	PD		PD		12
14	69	M	LN, lung, liver	M-VAC	PD		PD		5+
15	55	M	Liver	M-VAC	PR	3	PR	3	10+
16	73	M	LN, liver	MEC			PR	5	6+

M-VAC, methotrexate, vinblastine, doxorubicin, cisplatin; MEC, methotrexate, epirubicin, cisplatin; ITP, ifosfamide, paclitaxel, cisplatin; GC, gemcitabine, carboplatin; GDC, gemcitabine, docetaxel, carboplatin; LN, lymph node; PD, progressive disease; CR, complete response; PR, partial response; NC, no change

the efficacy with dose-intensive M-VAC schedules have prompted the identification of new agents active against TCC, such as the taxanes and gemcitabine. The overall response rates for two-drug regimens consisting of cisplatin-paclitaxel, carboplatin-paclitaxel, and cisplatin-gemcitabine range from 63% to 72%, 14% to 65% and 42% to 66%, respectively. The overall response rates for platinum-paclitaxel-gemcitabine three-drug regimens range from 58% to 80%.[7]

We administered two-drug and three-drug combinations of these new drugs as a pilot study in patients with metastatic urothelial carcinoma whose tumors were refractory to or had shown no response to platinum-based regimens.

Patients and methods

From August 1998 to May 2003, we treated 16 patients with metastatic urothelial cancer who had previously received one or more platinum-based regimens. The previous chemotherapies had been MEC[8] (methotrexate, epirubicin, cisplatin) in 11 patients, M-VAC[6] in 6, and ITP[9] (ifosfamide, paclitaxel, cisplatin) in 7 patients. Informed consent was obtained from all patients, and this clinical study was done with the Institutional Review Board's approval.

The patients were all men, with a mean age of 64 years (range, 46 to 73 years). Thirteen patients had bladder cancer and 3 had pelvic or ureteral cancer. The pathological diagnoses of the primary tumors were all grade II to III TCC. Two patients had already undergone radical cystectomy and 2 patients, nephroureterectomy. Metastases were limited to the lymph nodes in 4 patients; to the lymph node and lung in 1 patient; to the lymph nodes, lung, and bone in 1 patient; and to the lymph nodes, lung, and liver in 3 patients. Metastases were restricted to only the lung in 3 patients, to only the bone in 2, and to only the liver in 2 (Table 1).

Treatment

The treatment schedule for the combination of gemcitabine and carboplatin (GC) was gemcitabine 750 mg/m^2 over 30 to 60 min on days 1, 8, and 15; and carboplatin 200 mg/m^2 on day 2. Cycles were repeated every 28 days.[10] The treatment schedule for the comination of gemcitabine, docetaxel, and carboplatin (GDC) was gemcitabine 750 mg/m^2 on days 1 and 8, docetaxel 50 mg/m^2 on day 1, and carboplatin 200 mg/m^2 on day 1. Cycles were repeated every 21 days.[11,12] These treatments were carried out for a maximum of eight cycles of GC and six cycles of GDC in responding patients or patients with stable disease, but they were discontinued in the presence of disease progression. A detailed medical interview, clinical examination, and laboratory studies were obtained before each drug administration. Dose adjustment was based on assessment of the hematological and nonhematological toxicities. In particular, only 75% of the gemcitabine dose was administered when granulocytes measured 1.0–1.4 $\times$ 10^9/l and/or platelets were 75–99.9 $\times$ 10^9/l. If granulocytes were 0.5–0.9 $\times$ 10^9/l and/or platelets were 50–74.9 $\times$ 10^9/l, 50% of the full dose was administered. If the cell counts fell below the lower level of either range, further treatment was delayed until recovery.

GC was administered to 15 patients and GDC to 9 patients. Eight of the 9 GDC-treated patients had previously been treated with GC and had become refractory (Table 1). The median time from discontinuation of GC to the start of GDC in these 8 patients was 1 month. The median number and range of cycles of GC therapy were 3 and 2 to 8. The median number and range of cycles of GDC therapy were 3 and 1 to 6.

Evaluation of response and toxicity

All patients who completed at least one therapy cycle (three injections of gemcitabine and tumor reassessment after a 1-week interval) were analyzed for chemotherapeutic efficacy. All enrolled patients were analyzed for toxicity and survival and were reviewed every month to assess efficacy and toxicity. After discontinuation of treatment, patients were evaluated every month to assess the survival and disease-free status. The evaluation of the tumor response was based on the standard WHO criteria for measurable disease.[13]

For the evaluations of the tumor response and survival, the following definitions were used: time to response, the time from first injection to first objective response; time to progression, the time from first injection to the date of evidence of progression; time to treatment failure, the time from first injection to date of withdrawal from the study for any reason (progression, toxicity, refusal); duration of partial response (PR), the time from first evidence of PR to the time of disease progression; duration of complete response (CR), the time from first evidence of CR to the time of disease progression; and survival, the time from first injection to death.

Kaplan-Meier analysis was used for analysis of the survival and time to progression, and the 95% confidence interval (CI) was also calculated.

Results

Response

Fifteen patients received at least two courses of GC and 9 patients received at least one course of GDC, so only these patients were evaluated for response and toxicity. With the GC therapy, 7 of the 15 patients (47%; 95% CI, 21%–73%) showed an objective response, with 3 achieving a CR and 4, a PR. The time to response in all responders was within 2 months. With the GDC therapy, 6 of the 9 treated patients (67%; 95% CI, 29%–92%) showed an objective response, with 1 achieving CR and 5, PR. Five of the 6 responders were refractory to GC therapy, and in all 6 patients the time to response was 1 month. The median duration of response was 4 months (range, 2–10+ months) with GC and 3 months (range, 3–5 months) with GDC (Table 1). The median times to progression with the GC and GDC therapies were 4.5 months and 4 months, respectively. The median survival for all patients was 8 months.

All three CRs in patients on GC therapy occurred in the lymph nodes, while the one CR in the patient on GDC therapy occurred in the lung. The four PRs in patients on GC therapy occurred in the liver and lungs, while the PRs in patients on GDC therapy occurred in lungs in two patients and the liver in three patients.

Three of the six patients with liver metastasis achieved a PR. Patient 10 (patient number in Table 1) had multiple liver metastases, jaundice, and total bilirubin of 10 mg/dl when he was referred to our hospital. GC therapy was performed by hepatic arterial infusion by inserting an arterial infusion catheter into the femoral artery through a port set in the femoral subcutaneous area. PR was obtained within two cycles. After eight cycles; however, new liver metastasis appeared, and so we switched to GDC therapy. The new liver metastasis decreased, and PR was obtained again and continued for 5 months. His bilirubin level dropped to the normal range, and tumor markers[14] (carcinaembryonic antigen [CEA], carbohydrate [CA] 19-9, and CA125) also decreased to almost normal ranges. However, with these chemotherapies (eight cycles of GC and five cycles of GDC) computed tomography (CT) showed atrophy of liver without elevation of aspartate aminotransferase (AST)/alanine aminotransferase (ALT), or alkaline phosphatase (ALP), but endoscopy showed esophageal varices. We diagnosed the occurrence of liver cirrhosis caused by fibrotic change of massive metastatic liver tumor necrosis after chemotherapy. Accordingly, we terminated the chemotherapy, and the patient died 2 months later, with multiple liver metastases and ascites. In patient 15, the liver lesion responded to GC and GDC therapies and PRs were obtained and continued for 3 and 3 months, respectively. In patient 16, a 1.5-cm liver metastasis had appeared during the previous MEC therapy for pelvic lymph node metastases. The liver lesion responded to GDC therapy, and PR was obtained and continued for 5 months.

In patient 9, lung metastasis occurred during GC therapy but responded to GDC therapy; CR was obtained, but the duration was only 3 months. GDC therapy resulted in a PR of 3 months' duration in patients 8 and 11 with lung metastasis which had become refractory to GC therapy.

Toxicity

The treatments were generally well tolerated (Tables 2, 3). Grade 3 pancytopenia was observed in two patients treated with GC and in seven patients treated with GDC. The incidence of infection related to neutropenia was 11% (1/9) in patients on GDC therapy, with no WHO grade 3–4 infections. There were no cases of WHO grade 3–4 biochemical toxicity of AST/ALT, ALP, or bilirubin, and no transient elevation of AST, ALT, or ALP. No patients had WHO grade 3–4 elevation of the serum creatinine level or blood

Table 2. Toxicity of GC according to WHO toxicity scale(n = 15)

Toxicity	Grade			
	1 (%)	2 (%)	3 (%)	4 (%)
Neutropenia	0	0	7	7
Anemia	0	0	7	7
Thrombocytopenia	0	0	13	0
Neuropathy	0	0	0	0
Myalgia	0	0	0	0
Alopecia	0	0	0	0
Diarrhea	13	0	0	0

GC, gemcitabine, carboplatin; WHO, World Health Organization

Table 3. Toxicity of GDC according to WHO toxicity scale (n = 9)

Toxicity	Grade			
	1 (%)	2 (%)	3 (%)	4 (%)
Neutropenia	0	0	22	55
Anemia	0	0	55	22
Thrombocytopenia	0	0	33	33
Neuropathy	0	0	0	0
Myalgia	0	0	0	0
Alopecia	0	50	50	0
Diarrhea	22	11	0	0

GDC, gemcitabine, docetaxel, carboplatin; WHO, World Health Organization

urea nitrogen (BUN). With regard to symptomatic toxicity, nausea and vomiting were generally modest. Alopecia occurred in all GDC-treated patients.

Discussion

Transitional cell carcinoma (TCC) of the urothelium is a chemosensitive tumor, as demonstrated by its overall response rate of 35%–70% with the M-VAC drug combination.[6] The toxicity of this regimen, however, is significant, and the median survival of all treated patients does not greatly exceed 12 months.[15,16] These results have prompted a search for new active agents which could be incorporated into more effective and less toxic regimens.

Gemcitabine has been studied as a single agent for the treatment of metastatic bladder cancer,[3,17] and, based on its mechanism of action, it was thought to have potential for synergism with cisplatin. This synergism was later confirmed.[18]

Liver metastases generally do not respond well to M-VAC. On the other hand, liver metastases have a chance to respond to gemcitabine, as noted by Pollera et al.[17] They reported that three of seven patients with liver metastasis responded well to gemcitabine. Stadler et al.[3] also stated that three of nine patients with liver metastasis achieved a CR on gemcitabine monotherapy. One of our patients with multiple liver metastases survived for 15 months. When he was referred to our hospital, his total bilirubin was 10 mg/dl and jaundice was seen. Arterial infusion chemotherapy with GC was dramatically effective, and his bilirubin value dropped after two courses of GC. Almost the same effect was observed in patient 16. In another patient, a 1.5-cm liver metastasis had been found during MEC therapy for pelvic lymph node metastasis, but GDC therapy was effective, and a PR was obtained.

GC provides a survival advantage similar to that seen with M-VAC, while having a better safety profile and tolerability.[19] This better risk ratio should lead to a change in the standard of care for patients with metastatic TCC, with chemotherapy changed from M-VAC to GC.

Our results showed that some tumors which had become refractory to GC were still sensitive to GDC. Among our eight patients refractory to GC treatment, five (63%) responded to GDC. The responding organ was the lung in two patients and the liver in three patients. Therefore, we think GC is suitable as a first-line chemotherapy, and GDC is useable as a second-line chemotherapy. However, the durations of the response to GC and GDC therapies were short, so a new combination chemotherapy showing a longer response is desired. The metastatic urothelial tumors which became refractory to GC had a chance to respond to GC plus docetaxel (GDC chemotherapy). To our knowledge, this is the first published report showing that GDC is effective for GC-refractory metastatic urothelial tumors. It remains unclear whether, in order to prolong the chemotherapy effect, GC should be used as first-line chemotherapy, with GDC as second-line therapy; or whether GDC should be used as first-line chemotherapy. Accordingly, a double-blind randomized study of two arms, GC to GDC and GDC, is now in progress.

All of our patients had previously received cisplatin-based chemotherapy and radiotherapy. GC showed mild toxicity (patients experienced little WHO grade 3 toxicity), which was easily manageable. GC is usually well tolerated and is suitable for outpatient use. Neither unexpected nor cumulative toxic effects were found. Furthermore, the absence of significant renal and cardiac toxicities makes this promising combination especially attractive for urothelial cancer, allowing GC to be used in patients who may not be able to tolerate more toxic chemotherapeutic regimens.

We showed that metastatic urothelial tumors refractory to GC therapy still responded to GDC therapy. However, GDC therapy had the disadvantage of a short duration of response. Accordingly, another combination chemotherapy of gemcitabine with an epidermal growth factor receptor inhibitor is now being investigated.[20]

References

1. Hertel LW, Boder GB, Kroin JS, et al. (1990) Evaluation of the antitumor activity of gemcitabine (2′,2′-difluoro-2′-deoxycytidine). Cancer Res 50:4417–4422
2. Lorusso V, Pollera CF, Antimi M, et al. (1998) A phase II study of gemcitabine in patients with transitional cell carcinoma of the urinary tract previously treated with platinum. Italian Co-operative Group on Bladder Cancer. Eur J Cancer 34:1208–1212
3. Stadler WM, Kuzel T, Roth B, et al. (1997) Phase II study of single-agent gemcitabine in previously untreated patients with metastatic urothelial cancer. J Clin Oncol 15:3394–3398
4. Vaishampayan U, Parchment RE, Jasti BR, et al. (1999) Taxanes: an overview of the pharmacokinetics and pharmacodynamics. Urology 54 (Suppl 6A):22–29
5. Haldar S, Basu A, Croce CM (1997) Bcl2 is the guardian of microtubule integrity. Cancer Res 57:229–233
6. Sternberg CN, Yagoda A, Scher HI, et al. (1989) Methotrexate, vinblastine, doxorubicin, and cisplatin for advanced transitional cell carcinoma of the urothelium. Efficacy and patterns of response and relapse. Cancer 64:2448–2458
7. Bellmunt J, Guillem V, Paz-Ares L, et al. (2000) Gemcitabine/paclitaxel-based three-drug regimens in advanced urothelial cancer. Eur J Cancer 36 (Suppl 2):17–25
8. Kuroda M, Kotake T, Akaza H, et al. (1998) Efficacy of dose-intensified MEC (methotrexate, epirubicin and cisplatin) chemotherapy for advanced urothelial carcinoma: a prospective

randomized trial comparing MEC and M-VAC (methotrexate, vinblastine, doxorubicin and cisplatin). Japanese Urothelial Cancer Research Group. Jpn J Clin Oncol 28:497–501
9. Bajorin DF, McCaffrey JA, Dodd PM, et al. (2000) Ifosfamide, paclitaxel, and cisplatin for patients with advanced transitional cell carcinoma of the urothelial tract: final report of a phase II trial evaluating two dosing schedules. Cancer 88:1671–1678
10. Lorusso V, Manzione L, De Vita F, et al. (2000) Gemcitabine plus cisplatin for advanced transitional cell carcinoma of the urinary tract: a phase II multicenter trial. J Urol 164:53–56
11. Pectasides D, Glotsos J, Bountouroglou N, et al. (2002) Weekly chemotherapy with docetaxel, gemcitabine and cisplatin in advanced transitional cell urothelial cancer: a phase II trial. Ann Oncol 13:243–250
12. Hussain M, Vaishampayan U, Du W, et al. (2001) Combination paclitaxel, carboplatin, and gemcitabine is an active treatment for advanced urothelial cancer. J Clin Oncol 19:2527–2533
13. World Health Organization (1979) WHO Handbook for reporting results of cancer treatment. WHO, Geneva
14. Cook AM, Huddart RA, Jay G, et al. (2000) The utility of tumour markers in assessing the response to chemotherapy in advanced bladder cancer. Br J Cancer 82:1952–1957
15. Tannock I, Gospodarowicz M, Connolly J, et al. (1989) M-VAC (methotrexate, vinblastine, doxorubicin and cisplatin) chemotherapy for transitional cell carcinoma: the Princess Margaret Hospital experience. J Urol 142:289–292
16. Dimopoulos MA, Finn L, Logothetis CJ (1994) Pattern of failure and survival of patients with metastatic urothelial tumors relapsing after cis-platinum-based chemotherapy. J Urol 151: 598–600
17. Pollera CF, Ceribelli A, Crecco M, et al. (1994) Weekly gemcitabine in advanced bladder cancer: a preliminary report from a phase I study. Ann Oncol 5:182–184
18. Peters GJ, Bergman AM, Ruiz van Haperen VW, et al. (1995) Interaction between cisplatin and gemcitabine in vitro and in vivo. Semin Oncol 22 (Suppl 11):72–79
19. von der Maase H, Hansen SW, Roberts JT, et al. (2000) Gemcitabine and cisplatin versus methotrexate, vinblastine, doxorubicin, and cisplatin in advanced or metastatic bladder cancer: results of a large, randomized, multinational, multicenter, phase III study. J Clin Oncol 17:3068–3077
20. Stadler WM (2002) Gemcitabine doublets in advanced urothelial cancer. Semin Oncol 29 (Suppl 3):15–19

European Urology

European Urology 45 (2004) 176–181

Clinical Outcome of a Large-Scale Multi-Institutional Retrospective Study for Locally Advanced Bladder Cancer: A Survey Including 1131 Patients Treated during 1990–2000 in Japan

Hiroyuki Nishiyama[a], Tomonori Habuchi[a], Jun Watanabe[a], Satoshi Teramukai[b], Harue Tada[b], Yoshinari Ono[c], Shinichi Ohshima[c], Kiyohide Fujimoto[d], Yoshihiko Hirao[d], Masanori Fukushima[b], Osamu Ogawa[a,*]

[a]*Department of Urology, Kyoto University, Graduate School of Medicine, 54 Shogoin Kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan*
[b]*Translational Research Center, Kyoto University Hospital, Kyoto, Japan*
[c]*Department of Urology, Nagoya University, Graduate School of Medicine, Nagoya, Japan*
[d]*Department of Urology, Nara Medical University, Nara, Japan*

Accepted 12 September 2003

Published online 14 October 2003

Abstract

Objective: We conducted a multi-institutional analysis to establish the contemporary clinical outcome of invasive bladder cancer treated with radical cystectomy in Japan.

Methods: A total of 1131 consecutive patients who underwent radical cystectomy for invasive bladder cancer between January 1990 and December 2000 at 32 hospitals were retrospectively analyzed.

Results: Histopathological analysis demonstrated that 1042 patients (92.1%) harbored transitional cell carcinomas (TCCs), whereas 89 patients (7.9%) presented non-TCCs, including squamous cell carcinoma and adenocarcinoma. Pelvic lymphadenectomy was performed in 1013 patients in total, and pathologically confirmed lymph node metastases were found in 162 (16.0%). The overall survival at 5 years was 68.0% and most deaths (79.0%) occurred within 3 years. Multivariate analysis demonstrated that gender, clinical stage, pathological stage, lymph node involvement and lymph node dissection were the independent predictive factors for survival, whereas histological type, sex and grade had no siginificant impact on survival.

Conclusions: These clinical results demonstrate that radical cystectomy with lymph node dissection results in good survival for invasive bladder cancer, providing standard data with which other forms of therapy can be compared.

Keywords: Bladder cancer; Radical cystectomy

1. Introduction

Bladder cancer is the second most common genitourinary malignancy, which comprises a broad spectrum of tumors, including transitional cell carcinomas (TCCs), squamous cell carcinomas (SCCs), adenocarcinomas, small cell carcinomas, and miscellaneous subtypes [1,2]. TCCs are the most common histological subtypes and represent nearly 90% of all bladder cancers in Western countries, and 20% to 40% of bladder cancers present with or develop invasive disease. Invasive bladder cancers are very aggressive and have poor prognosis with fewer than 15% of TCC patients surviving 2 years if untreated [3]. During the last two decades, radical cystectomy with lymph node (LN) dissection has emerged as one of the standard forms of therapy for patients with invasive bladder cancer [4–7]. Early results of radical cystectomy suggests that only about 40% of patients treated for

* Corresponding author. Tel. +81-75-751-3325; Fax: +81-75-761-3441.
E-mail address: ogawao@kuhp.kyoto-u.ac.jp (O. Ogawa).

0302-2838/$ – see front matter
doi:10.1016/j.eururo.2003.09.011