Elevated Ki-67 (MIB-1) Expression as an Independent Predictor for Poor Prognosis After Radical Cystectomy for Bladder Cancer

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Purpose: We tried to investigate the clinical impact of Ki-67 (MIB-1) expression on the oncological and survival outcomes in patients with bladder cancer (BCa) after the radical cystectomy.

Materials and Methods: We retrospectively analyzed the data of 230 patients who were treated by radical cystectomy for BCa. Multivariate Cox-proportional hazards models and logistic regression tests were performed to evaluate the prognostic value of each variable.

Results: The patients with positive Ki-67 expression showed significant worse clinical characteristics and pathologic outcomes than negative Ki-67 group. Furthermore, the patients with Ki-67 expression showed significant worse recurrence ($p=0.018$) and cancer-specific mortality free survival ($p=0.019$) than negative Ki-67 group. The overall survival was also revealed to be inferior in Ki-67 positive group than Ki-67 negative group but the statistical significance was marginal ($p=0.062$). Subsequent multivariate Cox analyses showed that Ki-67 was independent predictor for disease recurrence after surgery (hazard ratio, 3.142; 95% CI, 1.287–7.671; $p=0.012$).

Conclusions: In our study, high Ki-67 expression was significantly related with worse clinical outcomes after radical cystectomy in the patients with BCa. Further prospective and basic researches are needed to validate the true prognostic value of Ki-67. (Korean J Urol Oncol 2017;15:152-157)

Key Words: Bladder cancer • Cystectomy • Ki-67 • Survival • Recurrence

INTRODUCTION

Bladder cancer (BCa) is the 9th most common malignancy and approximately 430,000 patients were diagnosed as BCa only in 2012.¹ BCa is also well-known to occur in the under-

developed countries and to have strong association with male gender. The risk factors for the having BCa include Schistosoma haematobium infection, tobacco smoking, chemical exposures, and diabetes mellitus treated by pioglitazone.²⁻⁴ The current gold-standard treatment is the radical cystectomy for the patients with muscle invasive BCa (MIBC) or high-risk recurrent nonmuscle invasive BCa (NMIBC).^{5,6} However there are no accurate biomarkers to predict the patients' prognosis after surgery, which is crucial for the patients' counseling and determining the treatment plan.

The expression of Ki-67 protein is known to be strictly associated with cell proliferation.⁷ The Ki-67 protein is present only in the mitosis stage and absent in the resting stage. This fact

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makes Ki-67 expression as an excellent biomarker to identify the status of cell proliferation. The fraction of Ki-67 positive cancer cells is often utilized to evaluate the clinical prognosis of the corresponding disease.⁸ However, there had been not many studies to identify the prognostic value of Ki-67 in the BCa cohort. Therefore we tried to evaluate the clinical usefulness of Ki-67 expression in our institutional BCa cohorts which was treated by radical cystectomy for BCa. We tried to evaluate the clinical correlation between the expression of Ki-67 upon postoperative survival outcomes in terms of recurrence-free survival (RFS), cancer-specific survival (CSS), and overall survival (OS) in our study.

MATERIALS AND METHODS

After the approval of Seoul National University Ethical Review Board (L-2017-1370), we analyzed the data of 29 patients who underwent radical cystectomy from December 2005 to December 2016 at Seoul National University Bundang Hospital. After additional exclusion of patients (preoperative radiotherapy [n=4], other malignancies [n=12], and incomplete information [n=3]), we finally analyzed a total of 230 patients. Patients' clinical and pathological information were acquired from our database which is prospectively maintained. All surgical specimens were reviewed by senior pathologist who was to-

tally unaware of clinical information about patients. The TNM staging was classified by using the 6th edition of the American Joint Committee cancer guidelines for BCa.⁹ The pathologic results including immunohistochemistry were also collected prospectively but analyzed retrospectively. The postoperative follow-ups were usually performed at 3- to 6-month intervals at the initial 2 years and yearly thereafter. Recurrence of disease was defined as radiologic or pathologic evidence of local recurrence, distant metastasis, or mortalities from BCa. Postoperative evaluations were generally performed by 3 to 6 months intervals for the first 2 years and yearly thereafter but slightly differ between the attending clinicians. Mortality data was acquired from the official database of the Korean National Statistical Office and also validated by reviewing our medical records. The RFS, CSS, and OS were determined from surgery date to progression, any-cause mortality, or cancer-specific mortality, respectively.

1. Immunohistochemical Assay

Specimens were fixed in 20% formalin and embedded in paraffin. The specimen was section by 5-mm interval and deparaffinized in xylene and rehydrated in ethanol. Hematoxylin and eosin staining and Ki-67 staining was performed. Ki-67 immunohistochemistry staining was performed by the streptavidin-biotin technique using the Ki-67 antibody (MIB-1, DAKO

Table 1. Summarization of clinical and pathologic characteristics of entire patients

Characteristic	Entire patients (n=230)	Ki-67 positive (n=186)	Ki-67 negative (n=44)	p-value
Age (y)	67.0 (59.8–74.2)	67.0 (60.0–75.0)	68.5 (56.0–73.0)	
Male sex	198 (86.1)	161 (86.6)	37 (84.1)	0.634
Body mass index (kg/m ²)	23.8 (21.7–25.5)	23.8 (21.7–25.5)	23.8 (21.7–25.9)	
Diabetes mellitus, yes	42 (18.3)	34 (18.3)	8 (18.2)	0.988
Hypertension, yes	91 (39.6)	72 (38.7)	19 (43.2)	0.610
ECOG score, ≥ 1	100 (43.5)	85 (45.7)	15 (34.1)	0.179
Highest TUR stage				0.024
Any stage/CIS	16 (7.0)	9 (4.8)	7 (15.9)	
T1	90 (39.1)	72 (38.7)	18 (40.9)	
T2	124 (53.9)	105 (56.5)	19 (43.2)	
Preoperative hydronephrosis, yes	77 (33.5)	68 (36.6)	9 (20.5)	
Neoadjuvant chemotherapy, yes	42 (18.3)	28 (15.1)	14 (31.8)	0.016
Pathologic stage				0.003
≤pT1	58 (25.2)	38 (20.4)	20 (45.5)	
pT2	86 (37.4)	73 (39.2)	13 (29.5)	
≥pT3	86 (37.4)	75 (40.3)	11 (25.0)	

Values are presented as median (range) or number (%).

ECOG: Eastern Cooperative Oncology Group, TUR: transurethral resection, CIS: carcinoma *in situ*.

Carpinteria, CA, USA). The deparaffinization of specimen sections was placed into Peroxidase Blocking Solution (DAKO) for 5 minutes, and incubation was performed with primary antibody of Ki-67 for 1 hour. After incubation, sections were counterstained by hemallume and the percentage of positive nuclear staining by Ki-67 was evaluated. The positive expression was defined when there were Ki-67 positive cells more than 10% percentage of entire tumor cells, and the others were defined to have negative expression.

2. Statistical Analyses

The independent t-tests and chi-square tests were performed to compare the clinicopathological characteristics between the groups. Cox proportional hazard models were utilized to evaluate possible clinical associations on each survival end-points. All of the statistical analyses were performed by using IBM SPSS Statistics ver. 19.0 (IBM Co., Armonk, NY, USA). All p-values were presented as two-sided and $p < 0.05$ was considered to be statistically significant.

RESULTS

The clinical and pathologic characteristics of patients were summarized in Table 1. The median age at surgery was 67.0 years (interquartile range [IQR], 59.8–74.2 years) and median duration of follow up was 26.3 months (IQR, 7.9–60.7 months). There were 186 patients (80.9%) with positive Ki-67 expression and 44 patients (19.1%) without Ki67 expression. The patients with positive Ki-67 expression had significantly worse previous TUR stage ($p=0.024$) and also pathologic stages ($p=0.003$). However there were no significant differences in other clinical or pathologic characteristics. The Kaplan-Meier analyses revealed that positive Ki-67 group experienced significantly inferior RFS ($p=0.018$) and CSS ($p=0.019$) than the negative Ki-67 group (Fig. 1). The positive Ki-67 group showed high OS than Ki-67 negative group but did not reach the statistical significance. Subsequently, the multivariate Cox proportional analyses showed that the Ki-67 expression was an independent

Fig. 1. Kaplan-Meier analyses for recurrence-free (A), overall (B), and cancer-specific survivals (C) after radical cystectomy in patients with bladder cancer.

Table 2. Multivariate analyses using Cox proportional hazard model on recurrence-free, overall, and cancer-specific survivals after surgery

Variable	Recurrence-free survival			Overall survival			Cancer-specific survival		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
Age	0.975	0.953–0.998	0.031	1.027	0.988–1.067	0.182	1.009	0.963–1.057	0.716
Body mass index	1.060	0.971–1.156	0.193	0.997	0.874–1.137	0.965	1.007	0.855–1.186	0.931
Diabetes mellitus	1.158	0.594–2.256	0.666	1.810	0.796–4.116	0.157	2.113	0.821–5.439	0.121
Ki-67	3.142	1.287–7.671	0.012	3.072	0.691–13.662	0.140	21,820.9	0.000–6.580	0.878
ECOG score, ≥ 1	1.648	0.966–2.809	0.142	2.446	1.154–5.184	0.020	2.166	0.877–5.350	0.094
Hydronephrosis, yes	1.611	0.972–2.671	0.065	1.327	0.663–2.657	0.425	1.360	0.585–3.162	0.475
Pathologic stage									
$\leq pT1$		Reference			Reference			Reference	
pT2	2.688	1.101–6.560	0.030	6.377	0.785–51.771	0.083	22,700.5	0.000–2.098	0.894
$\geq pT3$	4.918	2.017–11.989	<0.001	16.515	2.113–129.1	0.008	60,499.7	0.000–5.586	0.884
Neoadjuvant chemotherapy, yes	4.053	2.088–7.868	<0.001	4.363	1.647–11.556	0.003	3.850	1.045–14.2	0.043
Pathologic cellular grade	0.472	0.054–4.156	0.499	11,514.8	0.000–999.9	0.987	0.516	0.000–1.994	0.998

HR: hazard ratio, CI: confidence interval, ECOG: Eastern Cooperative Oncology Group.

predictor for worse RFS after surgery (HR, 3.142; 95% CI, 1.287–7.671; $p=0.012$) but not in the OS and CSS (Table 2). The pathologic stage was also revealed as significant prognostic factor for recurrence free survival and OS. Moreover, the patients' age and neoadjuvant chemotherapy was showed significant results in the multivariate Cox analysis.

DISCUSSION

In this study, we observed that the high Ki-67 was significantly related with the worse survival outcomes after radical cystectomy in patients with BCa in terms of RFS and CSS. Moreover the expression of Ki-67 was revealed to be independent predictor of worse RFS from the multivariate Cox analysis. Even though the Ki-67 expression did not show significant association with CSS and OS in the multivariate analyses, we think that the statistical insignificance of our results is due to our study's small number of cohort. Therefore we believe that the Ki-67 expression should be re-evaluated in the further prospectively organized studies with more large number of subjects.

The Ki-67 protein is not a novel biomarker and its appearance is go back to 1980's. Gerdes et al.¹⁰ first demonstrated the Ki-67 and initial prototype monoclonal antibody, which was produced by tumor cells of Hodgkin lymphoma cell from mouse. As the Ki-67 expression can be observed in the all proliferating cells (normal and tumor cells), the Ki-67 expression was utilized as a biomarker which can represent the status of

cell proliferation. Subsequently, numerous studies tried to evaluate the prognostic value of Ki-67 in the various types of cancers.¹¹ Drach et al.¹¹ reported that the expression of Ki-67 is well-correlated with the course of the disease and has further strength in distinguishing multiple myeloma from other monoclonal gammopathy. Huuhtanen et al.¹² analyzed 123 patients with soft tissue sarcoma and concluded that the Ki-67 score was strong predictor for predicting the metastasis-free survival and CSS. Several group also showed that the Ki-67 expression can have excellent power in predicting the patients' prognosis in prostate cancer.^{13–15} Bai et al.¹³ analyzed small cohorts of 39 patients and found that the Ki-67 expression have prognostic information from their multivariate analyses. Moul et al.¹⁵ performed the immunohistochemistry staining using the pathologic specimens from 162 patients with localized prostate cancer and reported that Ki-67, p53, and bcl-2 have significant correlation with biochemical recurrence after surgery.

Preceding the present study, there had been several studies which tried to elucidate the clinical usefulness of Ki-67 in the patients with BCa.¹⁶ Margulis et al.¹⁶ analyzed the data of 226 patients with BCa. The Ki-67 expression was found in the 42.5% of cystectomy specimens and in 54% of metastatic lymph nodes. The Ki-67 was also significantly associated with the advanced pathologic stage ($p=0.001$), higher grade ($p=0.040$), and lymph node invasion ($p=0.036$) in their study. More recently, Wang et al.¹⁷ also analyzed the small cohort of 103 patients who were treated with radical cystectomy for BCa in single institution in china. They reported that there was sig-

nificant association between the Ki-67 expression and CSS in the female BCa patients. Apart from the MIBC, Ding et al.¹⁸ evaluate the association of Ki-67 and disease prognosis in patients with NMIBC. After analyzing the clinical data of 332 patients after transurethral resection, the Ki-67 expression was significantly associated with high European Organization for Research and Treatment of Cancer risk scores for tumor recurrence and progression. Furthermore, multivariate analyses revealed that Ki-67 was independent risk factor for tumor recurrence and progression of disease.

Our study is limited by its retrospective design though our database was managed prospectively. We also admit the possibility of selection bias since only patients who underwent radical cystectomy were included in our study. Moreover, the indication for neoadjuvant chemotherapy was not controlled and varies according to the physicians and time periods, which may significantly impact the outcomes of our study. However, our study is the first study which tried to evaluate the clinical significance of Ki-67 in Korean population. Further study with prospective design and large number of subjects are needed to evaluate the true clinical usefulness of Ki-67 expression in BCa patients.

CONCLUSIONS

The expression of Ki-67 was significantly associated with worse pathologic and survival outcomes in patients with localized prostate cancer. These findings may help the future clinicians to predict the PCa prognosis more exactly and to choose the optimal treatments.

CONFLICT OF INTEREST

The authors claim no conflicts of interest.

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