PROLIFERATIVE STATUS IS A RISK INDEX FOR RECURRENCE IN PRIMARY SUPERFICIAL (pTa/T1) LOW-GRADE UROTHELIAL BLADDER CARCINOMA

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The current clinicopathologic study for evaluation of superficial bladder cancer still has limitations in predicting the true behavior of recurrence. To determine the high-risk recurrence factors, we studied the influence of Ki-67, c-erbB-2, p53 and multidrug resistance-associated protein (MRP) expression. Samples were obtained from 33 pTa and 46 pT1 diagnosed bladder cancer patients with a mean follow-up of 48.7±30.6 months. The contingency table method, Kaplan-Meier curve and multivariate analysis were used to evaluate the association among the immunohistochemical factors expression, clinicopathologic parameters with tumor recurrence. Stage pT1 tumors, sessile tumors and large tumors (>3 cm) showed a significantly high recurrence rate ($p=0.0158$, $p=0.0162$, $p=0.0001$ respectively). Tumors with overexpression of Ki-67, c-erbB-2 and p53 were more likely to recur ($p=0.0035$, $p=0.0027$, $p=0.0076$ respectively), MRP expression was not associated with recurrence. Multivariate analysis showed that large tumors and high Ki-67 expression were independent indicators of recurrence. On the other hand, in tumors less than 1 cm, recurrence was significantly correlated with overexpression of Ki-67 and p53. High Ki-67 expression could discriminate higher recurrence cases in grade 2, pT1 and single tumors. The c-erbB-2 overexpression was more frequently associated with recurrence in sessile tumors, large tumors, multiple and grade 1 tumors. The p53 overexpression also predicted a higher risk of recurrence in pTa tumors. These data demonstrated that the use of proliferative related proteins yields significant prognostic information in addition to clinicopathological factors, high Ki-67 expression is a reliable indicator of recurrence. A combination rather than any factor alone could more accurately predict tumor recurrence.
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Key words: Ki-67, c-erbB-2, p53, Superficial bladder cancer, Recurrence

INTRODUCTION

Patients with primary superficial (pTa/pT1) bladder cancer are commonly treated by transurethral resection (TUR), and usually a good prognosis is expected. However, more than half of the patients have tumor recurrence and a potentiality of developing relapse[1,2] When the tumor infiltrates to the muscularis, about 78% of the patients eventually succumb to the disease[1]. It is important to search for valuable factors associated to high-risk of recurrence for early diagnosis and treatment. Since there still resides disagreement with traditional criteria[2–4], additional prognostic factors are necessary. In this study, we examined the expression of Ki-67 antigen, c-erbB-2 oncogene, p53 tumor suppressor gene and multidrug resistance-associated protein (MRP) by immunostaining in primary superficial low-grade (grade 1 and grade 2) urothelial (transitional cell) bladder carcinoma, and examined the correlation between overexpression of these gene products and the clinicopathological characteristics with tumor recurrence.

MATERIALS AND METHODS

1. Patients and the clinicopathological characteristics

The subjects were 79 patients (66 males and 13 females) with primary superficial low-grade urothelial bladder carcinoma who were treated by TUR and histologically confirmed at Mie University hospital. They had a median age of 64.1±13.0 years (range, 34–91 years). Seventy-four patients received intravesical anticancer drug (Mitomycin C and cytosine arabinoside or Adriamycin) treatment after TUR. Patients were followed for a mean of 48.7± 30.6 months (range 4–78 months). All patients were followed by cystoscopy every 3 months in the first and second year, every 4 months in the third year, every 6 months in the fourth and fifth year and annually thereafter if there was no recurrence. The recurrence time was calculated from the time of the initial TUR

Table 1. Association between the Ki-67 expression and the clinicopathological characteristics in superficial low-grade bladder cancer

Prognostic factors	Patients (%)	Ki-67 expression		P-value
		<18% (%)	≧18% (%)	
Grade				
1	23 (29)	18 (78)	5 (22)	0.021
2	56 (71)	28 (50)	28 (50)	
Stage				
pTa	33 (42)	23 (70)	10 (30)	0.08
pT1	46 (58)	23 (50)	23 (50)	
Morphology				
Pedicle	56 (71)	35 (63)	21 (37)	0.23
Sessile	23 (29)	11 (48)	12 (52)	
Size (cm)				
<1	20 (25)	16 (80)	4 (20)	
1–3	45 (57)	19 (42)	26 (58)	0.0115
>3	14 (18)	11 (79)	3 (21)	
Multiple				
No	43 (54)	26 (61)	17 (39)	0.66
Yes	36 (46)	20 (56)	16 (44)	
Immunostaining				
p53				
<20%	41 (52)	26 (64)	15 (36)	0.33
≧20%	38 (48)	20 (53)	18 (47)	
c-erbB-2				
<25%	43 (54)	30 (70)	13 (30)	0.023
≧25%	36 (46)	16 (44)	20 (56)	
MRP				
<25%	41 (52)	25 (61)	16 (39)	0.61
≧25%	38 (48)	21 (53)	17 (47)	

until the first recurrence by a positive cystoscopy with a biopsy-proven lesion. These patients did not show progress defined as "the presence of muscle invasion, metastasis, or death by bladder cancer" [4]. Tissue samples, stage and grade were reviewed and determined according to the general rules for clinical and pathological studies on bladder cancer[5]. The clinicopathological characteristics of patients are shown in Table 1.

2. Immunohistochemistry

All immunohistochemical analyses were performed on routinely processed, formalin-fixed, paraffin-embedded tissues employing a streptavidin-biotin method. Successive three micrometer tissue sections were cut from blocks selected for the presence of representative tumor tissue. Sections were deparaffinized and rehydrated. Briefly, endogenous peroxide was blocked by 0.3% hydrogen peroxide for 15 minutes, and washed twice with phosphate-buffered saline (PBS). Sections were placed in citric acid buffer (10 mM, pH 6.0) heated in a microwave oven (500 W) for 5 successive periods of 3 minutes, for antigen activation (for c-erbB-2, this process was unnecessary), and allowed to cool in citrate buffer for 20 minutes at room temperature. The sections were blocked with a super-block (ScyTek stain kit, ScyTek laboratories, USA) for 8 minutes at room temperature. Sections were incubated overnight at 4°C in a high-humidity chamber with primary antibody Ki-67 antigen (clone MIB-1, 1 : 50 dilution; Immunotech, Marseilles, France), c-erbB-2 oncoprotein (clone CB-11, 1 : 40 dilution; Novocastra laboratories, Newcastle, UK), p53 protein (clone DO-7, 1 : 25 dilution; Novocastra laboratories, Newcastle, UK), and MRP (clone MRPm6, 1 : 20 dilution; Progen Biotechnik gmbh, Germany. All dilutions of primary antibody were in 1% bovine serum albumin/PBS). Samples were incubated with biotinylated link antibody (ScyTek stain kit, USA) for 20 minutes at room temperature, and were incubated with streptavidin/HRP Label (ScyTek stain kit, USA) for 20 minutes at room temperature. After the above reaction ended, the peroxidase reaction was performed using a solution 3, 3'-diaminobenzidine (ScyTek stain kit, USA) as chromogen substrate for 5 minutes. Finally, the slides were lightly counterstained by hematoxylin.

A section of breast carcinoma for c-erbB-2, and a liver carcinoma with known positive immunstaining for p53 served as a positive control. Positive and negative control slides were included within each batch of slides. The intensity was scored using the

following system: 1+, those tumor cells showing equivocal staining; 2+, unequivocal with moderate intensity; and 3+, those tumor cells that showed strong staining. Tumor cells with 2+ or 3+ were considered as positive. For Ki-67 and p53 immunostaining of nucleus of tumor cells, and for c-erbB-2 and MRP, the staining of the cell membrane was regarded as positive. At least one thousand tumor cells were examined at a ×400 magnification considering the best stained field for each sample, the median value of positive cells percentage for labeling index were: for Ki-67, 18%; for p53, 20%; for c-erbB-2 and MRP, 25%. Above these values, it was considered overexpressed, which was modified from a previous report[6-8]

3. Statistical analysis

The association between immunohistochemical factors (Ki-67, p53, c-erbB-2 and MRP) expression and clinicopathologic parameters (grade, stage, tumor morphology, size and tumors number) were evaluated by using Fisher's exact test. The chi-square test was used to examine whether overexpression of Ki-67, p53, c-erbB-2 and MRP could indicate the risk of recurrence according to each clinicopathological parameter. Recurrence-free survival curves were calculated using the Kaplan-Meier method, and the log-rank test was used for this analysis. Multivariate analysis was performed using Cox's proportional hazard model and identified as independent predictor of tumor recurrence. The calculations were completed by using the Stat View 5.0 software. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

1. Ki-67, c-erbB-2, p53 and MRP expression

The association between the Ki-67 expression and the clinicopathological characteristics of patients are presented in Table 1. The MIB-1 antibody gave a nuclear staining and 33 of the 79 (42%) cases displayed high Ki-67 expression ($\geq$18% positive nuclei), a high proliferative index. Compared to 5 of 23 (22%) grade 1 tumor, high Ki-67 expression in 50% of grade 2 (28 of 56 cases) was significantly high (p=0.021). For tumor stage, high Ki-67 expression in 10 of the 33 (30%) pTa and in 23 of the 46 (50%) pT1 tumors were observed, but the difference did not reach statistical significance (p=0.08). The correlation between high Ki-67 expression and size of tumor was significant (p=0.012), but in tumors more than 3 cm in diameter, the high Ki-67 expression rate was found in only 21% of the tumors (3 of 14 cases). The high Ki-67 index was also more frequent in c-erbB-2 overexpressed tumors (p=0.023). It was observed in 20 of the 36 (56%) with c-erbB-2 overexpression and in 13 of the 43 (30%) with low expression. No relationship was observed between high Ki-67 expression and the morphology of tumors (p=0.23), number of tumors (0.66), p53 expression (p=0.33) or MRP expression (p=0.61).

In the evaluation of c-erbB-2 expression, staining of only the cell membrane was regarded as positive. c-erbB-2 was overexpressed in 36 of the 79 (46%) primary superficial low-grade urothelial bladder carcinoma, in 9 of the 23 (39%) grade 1 tumors, and in 27 of 56 (48%) grade 2 tumors (p=0.46). c-erbB-2 staining was found in 12 of the 33 (36%) pTa tumors, 24 of the 46 (52%) pT1 tumors (p=0.16). c-erbB-2 was overexpressed in 14 of the 23 sessile tumors (61%), more frequently compared to 22 of the 56 (39%) pedicle tumors, but this association did not reach statistical significance (p=0.08). c-erbB-2 overexpression was found in 6 of the 20 (30%) tumors less than 1 cm in diameter, in 21 of the 45 (47%) tumors of 1–3 cm and in 11 of the 14 (78%) tumors more than 3 cm in diameter. Thus, increased expression of c-erbB-2 was more frequent in large tumors, but this association was not significant (p=0.136). c-erbB-2 overexpression had also a significant association with p53 overexpression (p=0.01), with positive staining for c-erbB-2 observed in 32% (13 out of 41) of p53 group with underexpressed group and in 23 of the 38 (61%) p53-overexpressed tumors. No correlation was found between c-erbB-2 expression and the number of tumors (p=0.85) or MRP expression (p=0.23).

p53 was overexpressed in 38 of the 79 (48%) patients, and was found in 9 of the 23 (39%) grade 1, 29 of the 56 (52%) grade 2 tumors, but the difference was not significant (p=0.3). No correlation was found between p53 expression and tumor stage (p=0.6). Similar to c-erbB-2 overexpression, p53 overexpression was related to tumor morphology (p=0.14), detected more frequent that sessile tumor (14 out of 23 cases, 61%) compared to pedicled tumors (22 out of 56 cases, 39%). p53 was also correlated with tumor size, being found in 7 of the 20 (35%) tumors less than 1 cm in diameter, 23 of the 45 (51%) tumors 1–3 cm in diameter and 8 of the 14 (57%) tumors more than 3 cm in diameter, but this association was not significant (p=0.37). No significant association was observed with the number of tumors (p=0.13) or expression levels of MRP (p=0.9).

MRP is a multidrug resistance-associated protein. A positive score was assigned when the cell membrane was stained. MRP was overexpressed in 38 of the 79 (48%) patients, MRP overexpression was found in 57% (26 out of 46) of the pT1 tumors, and more than 36% (12 out of 33) of the pTa tumors, but this association was not significant (p=0.08). Similarly c-erbB-2 was also related with tumor size, being found in 35% of the tumors less than 1 cm in diameter, 49% of the tumors in the range of 1–3 cm and 82% of

Table 2. Association between the tumor grade and stage

Stage	Patients (%)	Grade I (n=23)	Grade II (n=56)	P-value
pTa	33 (42)	14 (44)	19 (56)	0.027
pT1	46 (58)	9 (20)	37 (80)	

tumors more than 3 cm in diameter, but this association was not significant (p=0.1). No correlation was found between MRP expression and tumor grade (p=0.6), or the number of tumors (p=0.29).

2. Recurrence probability

In this study, 31 of the 79 patients (39%) showed tumor recurrence. The association between the tumor grade and stage was significant (p=0.027) (Table 2).

The 5-year cumulative probability of recurrence between grade 1 and grade 2 tumors was not significant (p=0.98). Recurrence in pT1 tumors was 50.7%, and significantly higher compared with 24.2% of pTa tumors (p=0.0158) (Fig. 1A). For tumor morphology, recurrence in sessile tumors was 60.9%,

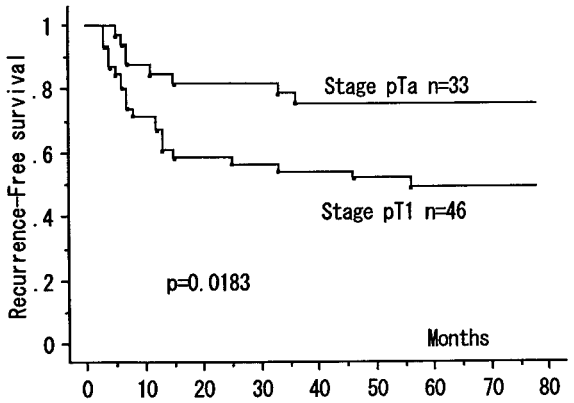

Fig. 1A. The 5-year cumulative probability of recurrence in stage pT1 tumors was 50.7%, and significantly higher compared with 24.2% of stage pTa (p=0.0158).

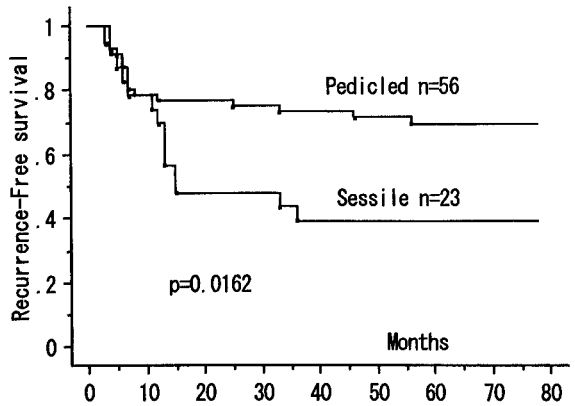

Fig. 1B. The 5-year cumulative probability of recurrence in sessile tumors was 60.9%, and significantly higher compared with 30.7% of pedicle tumors (p=0.0162).

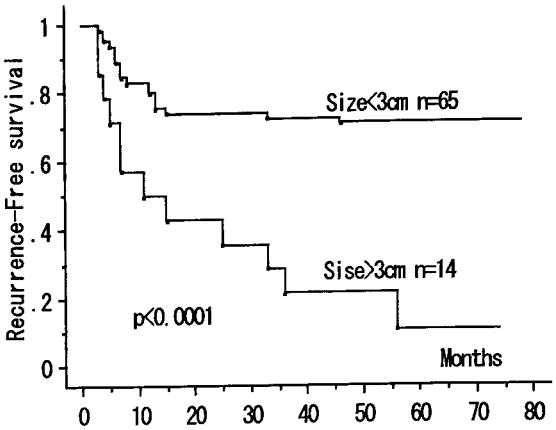

Fig. 1C. The 5-year cumulative probability of recurrence in tumors more than 3 cm in diameter was 89.3%, and significantly higher compared with 29.3% of those less than 3 cm (p=0.0001).

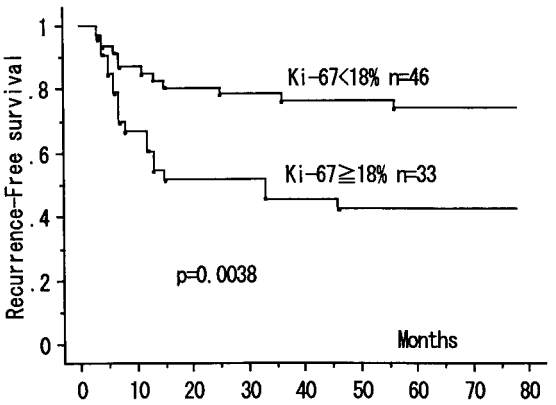

Fig. 2A. The 5-year cumulative probability of recurrence in the high Ki-67 expression group was 57.6%, and significantly higher compared with 26.4% in the low expression group (p=0.0038).

and significantly higher compared with 30.7% of pedicled tumors (p=0.0162) (Fig. 1B). Tumors size was significantly related to recurrence, especially tumors with a diameter more than 3 cm were more likely to recur than those less than 3 cm in diameter (p=0.0001) (Fig. 1C). Multiple tumors (44%) had a lower tendency to recur than single tumors (35.1%) (p=0.28).

The 5-year cumulative probability of recurrence with high Ki-67 expression was 57.6% (19 out of 33), and significantly higher than the 26.4% (12 out of 46) for tumors with low Ki-67 expression (p=0.0038) (Fig. 2A). For c-erbB-2, it was 58.9% (21 of the 36 cases) for overexpression c-erbB-2 also significantly correlated with a high risk of recurrence than the

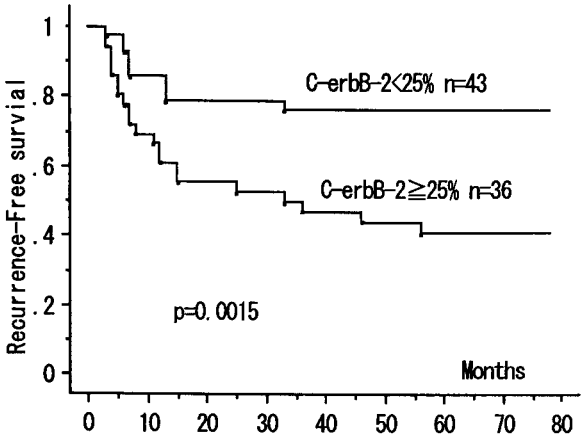

Fig. 2B. The 5-year cumulative probability of recurrence in c-erbB-2. Overexpression group was 58.9%, and significantly higher compared with 23.3% in the non-overexpression group (p=0.0015).

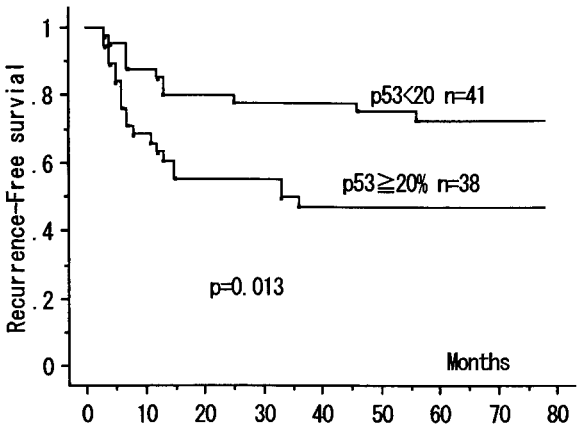

Fig. 2C. The 5-year cumulative probability of recurrence in p53 overexpression group was 52.6%, and significantly higher than 27.2% in the non-overexpression group (p=0.013).

Table 3. Multivariate analysis with Cox's proportional hazards regression test

Prognostic factors	Relative risk	95% confidence interval	P-value
p53 (≧20%/<20%)	1.92	0.84–4.38	0.124
c-erbB-2 (≧25%/<25%)	1.70	0.77–3.77	0.192
Ki-67 (≧18%/<18%)	2.68	1.23–5.84	0.014
Stage (pT1/pTa)	2.19	0.94–5.10	0.07
Morphology (Sessile/Pedicle)	1.16	0.51–2.61	0.71
Size (>3/<3 cm)	4.26	1.92–9.45	0.0004

23.3% (10 of the 43 cases) for non-overexpression (p=0.0015) (Fig. 2B). For p53 overexpression, it was 52.6% (20 out of 38 cases), and significantly higher than the 27.2% (11 of 41 cases) for non-overexpression (p=0.013) (Fig. 2C), but MRP expression did not correlate with recurrence.

When a Cox' proportional hazards model was constructed including tumor stage, size, morphology of tumor and Ki-67, c-erb-B-2, p53 expression, only large tumors (>3 cm) (p=0.0004; RR=4.26) and high Ki-67 expression (p=0.0135; RR=2.675) were independent predictors of tumor recurrence (Table 3).

3. Predictive potential of combined Ki-67, c-erbB-2 and p53 expression

In order to evaluate the potential cooperative effects of these markers, we combined the overexpression of Ki-67 and p53 or c-erbB-2 and evaluated whether this could more accurately predict tumor recurrence. Fourteen of the 31 (45%) recurrent tumors showed simultaneous overexpression of Ki-67 and p53, compared to only 4 of the 48 (8%) non-recurrent cases, this difference being significant (p<0.0001, log-rank test). A higher specificity (92%) is useful to accurately identify the high-risk of recurrence from superficial low-grade bladder cancers. Simultaneous overexpression of c-erbB-2 and Ki-67 was seen in 13 of the 31 (42%) recurrent tumors, and in 7 of the 48 (15%) non-recurrent tumors (p=0.002). Simultaneous overexpression of c-erbB-2 and p53 was seen in 14 of the 31 (45%) recurrent tumors, and in 9 of the 48 (19%) non-recurrent tumors (p=0.006).

4. Association between tumor recurrence and overexpression of Ki-67, c-erbB-2 and p53 according to each clinicopathological feature

We examined the predictive potential of the expression of these gene products according to each clinicopathological feature. Overexpression of c-erbB-2 in grade 1, and overexpression of p53 and Ki-67 in grade 2 were significantly associated with recurrence (p=0.0023, p=0.0116 and p=0.0062, respectively). Tumors with a high p53 expression had a significantly high recurrence rate in pTa tumor (RR=10.5, p=0.015), c-erbB-2 overexpression also had a higher recurrence, but was not statistically significant (RR=4.3, p=0.08). Recurrence of pT1 tumors correlated with increased expression of Ki-67 and c-erbB-2 (RR=5.2, p=0.008; RR=4.3, p=0.02, respectively). In pedicled tumors, high Ki-67 and c-erbB-2 expression showed a significant correlation with tumor recurrence (RR=3.6, p=0.03 and RR=3.2, p=0.05, respectively), for sessile tumors, the difference was also statistically significant with c-erbB-2 overexpression (RR=7.3, p=0.03). In small tumors (less than 1 cm), overexpression of p53 and Ki-67 were strongly correlated with recurrence (RR=16.0, p=0.015 and RR=21.0, p=0.01, respectively), and c-erbB-2 expression also showed a higher risk of recurrence (RR=6.0), but the difference

was not statistically significant (p=0.09). For 1–3 cm tumors only high Ki-67 expression predicted recurrence (RR=18.0, p=0.0014). In large tumors, recurrence was associated to c-erbB-2 overexpression (p=0.04). Overexpression of c-erbB-2 and p53 also correlated with recurrence in multiple tumors (RR=20.3, p=0.003, RR=6.7, p=0.0093, respectively). For single tumors, high Ki-67 expression showed a significantly high risk of recurrence (RR=3.8, p=0.04).

DISCUSSION

Clinicopathological recurrence risk factors for superficial bladder cancer include factors from the host (sex, age, job and smoking history); tumor factors (grade, stage, tumor morphology, tumor size and number); and treatment factors (oversight or imperfect excision of the tumor, sowing by operation, and supplementary treatment after operation). However, most investigators found in particular multiple tumors, more than 3 cm in diameter, stage pT1 and sessile tumor as the tumor factors[2–4] In our study, the recurrence rate was significantly high in large tumors, sessile and stage pT1 tumors. Though tumor grade was significantly correlated with stage and high Ki-67 expression, and was not related to recurrence in accordance with a previous report[9].

Cancer develops and evolves from multiple factors spaced as multiple temporal events. The clinicopathological features of tumors are phenotypes of many relevant genes. For example, histological grade is itself a composite, the features reflected in grade from many genes[10] Identification of gene expression patterns in superficial and invasive bladder cancer led us to a better understanding of those related to encoding proteins involved in cell proliferation, oncogenes and growth factors, cell adhesion, immunology, transcription, proteinases, and ribosomes[11] Recent studies have shown that multifocal bladder cancer and tumor recurrence have a monoclonal origin and intraepithelial spread of tumor cells or transplanted to bladder mucosa after TUR treatment[12,13] Therefore, tumor recurrence is also related to cell adhesion, proliferation and growth factors, and cell motility. We evaluated the predictive value for recurrence of tumors by expression of Ki-67, c-erbB-2, p53 and MRP combined with the clinicopathological features that should relate more closely to the event of recurrence in superficial low-grade bladder carcinoma.

Ki-67 antigen expressed in G1, S, G2 and M-phase determine the proliferate activity of the cell cycle. Many studies showed that the Ki-67 antigen correlated to grade, stage and other proliferation markers[6,7,10,14,15]. In superficial bladder cancer, high Ki-67 expression was correlated to recurrence of tumor, and was assumed to be an independent risk factor[7,14,15] It is well known that grade 2 bladder cancer is heterogeneous in biological potential, the high Ki-67 expression can discriminate grade 2 tumors with a favorable outcome[15], and was considered to be a valuable prognostic factor of recurrence as compared with grade and stage[7,14,15] The results of the present study were consistent with the above-mentioned studies, and using Ki-67 expression, we identified pT1 tumors with a high-risk of recurrence, even in small (<1 cm) and single tumors. Interestingly, in large tumors, only 3 out of 14 cases (21%) showed high Ki-67 expression, but all cases developed recurrence (100%), revealing a reduced expression rate of Ki-67 in large tumors. Bladder cancer is an angiogenic tumor, with evidence strongly linking microvessel density (MVD) to prognosis in solid tumors. The correlation is not so certain for papillary tumors[16] It is known that increased tumor size usually corresponds with an augmented MVD or broader tumor vessels. Based on the theory of differentiation inducement of cancer cells[17], when an efficient circulatory system is mandatory for all multicellular organisms to ensure the delivery of oxygen and nutrients, and aid the excretion of metabolic waste products with a "normal state of metabolism", the cancer cells become unstable by effects of various factors involved in tumor suppressor behavior, and is well or poorly differentiated. If the cancer cell has changed to a well-differentiated state[18,19], reduced Ki-67 expression also becomes possible in large tumors of primary superficial low-grade bladder cancer. However, this inference cannot explain the high-level expression of other simultaneously gene products in large tumors in this study, but reflects the disparate activity of various factors in cancer development.

c-erbB-2 is localized on chromosome 17q and encodes a glycoprotein with a molecular weight of 185,000[20] c-erbB-2 sensitizes tumor cells to mitogenic effects of heterologous growth factors by retarding degradation of liganded EGFR heterodimers[21] The activation of the intracellular kinase is thought to play a role in cell differentiation, motility and adhesion[22], an important step in recurrence, invasive and metastasis formation. The conflicts also still remain about the implication of c-erbB-2 expression[10,23,24] Previous reports showed that c-erbB-2 expression was able to predict recurrence in superficial bladder carcinoma[23,24] In our study, c-erbB-2 overexpression was significantly correlated with Ki-67 and p53 overexpression, thus the activating c-erbB-2 gene could lead to enhanced signals that inappropriately keep the cell going through the cell cycle. However, c-erbB-2 overexpression was not related to clinicopathological factors. Using univariate analysis, c-erbB-2 overexpression was significantly correlated to

Table 4. Association between tumor recurrence and Ki-67, c-erbB-2 and p53 overexpression according to each clinicopathological features

Prognostic factors	Total	Ki-67 (%)			c-erbB-2 (%)			p53 (%)		
		Recur	Non	RR	Recur	Non	RR	Recur	Non	RR
Grade										
1	33	3 (33)	2 (14)	3.0	7 (78)	2 (14)	21.0**	4 (44)	5 (36)	1.4
2	46	16 (73)	12 (35)	4.9**	14 (64)	13 (38)	2.8	16 (73)	13 (38)	4.3*
Stage										
Ta	23	3 (38)	7 (28)	1.5	5 (63)	7 (28)	4.3	7 (87)	10 (40)	10.5*
T1	56	16 (70)	7 (30)	5.2**	16 (70)	8 (35)	4.3*	13 (57)	8 (35)	2.4
Morphology										
Pedicled	56	10 (59)	11 (28)	3.6*	10 (59)	12 (31)	3.21*	9 (53)	13 (33)	2.3
Sessile	23	9 (64)	3 (33)	3.6	11 (73)	3 (33)	7.3*	11 (79)	5 (56)	2.9
Size (cm)										
<1	20	3 (60)	1 (7)	21.0**	3 (60)	3 (20)	6.0	4 (80)	3 (20)	16.0*
1–3	45	13 (93)	13 (42)	18.0*	9 (64)	12 (39)	2.9	9 (64)	14 (45)	2.19
>3	14	3 (25)	0	—	9 (75)	0	—*	7 (58)	1 (50)	1.4
Multiple										
No	41	9 (60)	8 (29)	3.8*	9 (60)	11 (39)	2.3	10 (67)	14 (50)	2.0
Yes	38	10 (63)	6 (30)	3.3	12 (75)	4 (20)	12.0**	10 (63)	4 (20)	6.7**

Recur: Recurrence, Non: Non-recurrence. RR: Relative risk. χ^2 test p-value: *<0.05, **<0.01.

tumor recurrence, especially in large tumors, sessile tumors and multiple tumors.

The p53 tumor suppressor gene has an important role in the regulation of normal cell growth and apoptosis. Mutations within the p53 gene result in loss of the ability of the p53 protein to cause growth arrest. Overexpression of p53 revealed by immunohistochemical assays correlates well with p53 mutations. The p53 overexpression was assumed to be related with the recurrence of superficial bladder cancer[25–27]. and to be the most important prognostic factor for the recurrence of bladder cancer[27] In our study, p53 overexpression was significantly correlated or related with tumor recurrence. We also showed that in patients with pTa, tumors less than 1 cm in diameter, the incidence of recurrence was high when p53 was overexpressed. p53 expression was significantly associated with an earlier first tumor recurrence[25]. One possible explanation can be that the allelic loss of p53 gene is associated with genesis and maintenance[28]

MRP overexpression is often responsible for the development of multidrug resistance in cancer therapy. These proteins are also expressed in normal tissues, where their physiological role is related to the extrusion of endogenous toxins or to secretory function in the liver and kidney[29] High MRP expression associated with an enhanced drug excretion was observed in human bladder cancer cell lines[30] We examined this protein expression using the MRPm6 monoclonal antibody that reacts with an internal epitope of MRP. MRP overexpression was not related to recurrence of tumors, but it increased in pT1 and large tumors, although this association was not significant.

In this study, among the clinicopathological factors, stage T1 tumors, sessile tumor and tumors more than 3 cm in diamater showed a significant high recurrence rate; for the immunohistochemical factors, tumors positive for Ki-67, c-erbB-2 and p53 overexpression were more likely to recur. Multivariate analysis showed that large tumors as well as high Ki-67 expression were independent prognostic indicators of recurrence. On the other hand, these results indicate that a combination of Ki-67, c-erbB-2, and p53 expression profile may be a better prognostic indicator than any factor alone, that could more accurately predict tumor recurrence. Evaluation of the recurrence probability of tumors by Ki-67, c-erbB-2 and p53 overexpression according to each clinicopathological factors, help to identify patients who are at risk for recurrence and might benefit from a selective aggressive adjuvant treatment while sparing low-risk patients from unnecessary therapies in superficial low-grade bladder cancers (Table 4). These data further demonstrate that proliferative related proteins yield significant prognostic information in addition to tumor stage and grade and other clinicopathological factors.

In conclusion, high Ki-67 expression was a reliable indicator of recurrence, but a combination of multiple factors analyses will be better than any factor alone, leading to a more accurate predicting of recurrence tumors. Additional studies are needed to elucidate the further validity of this observation, and the role of these factors in the evaluation of superficial low-grade bladder tumors.

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和文抄録

初発の表在性低悪性度の膀胱癌においては，増殖状態が再発危険因子である

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表在性膀胱癌の再発を予測する上で，臨床病理学的研究には，まだ限界がある．再発危険因子検索のため，われわれは pTa 33例と pT1 46例の膀胱癌患者に Ki-67，c-erbB-2，p53 と多薬剤耐性蛋白（MRP）を免疫組織化学的に検討した．追跡調査期間は平均48.7±30.6カ月だった．臨床病理学因子，免疫組織化学因子および腫瘍再発の関係については，分割表方法，Kaplan-Meier 曲線，多変量解析によって解析した．PT1，広基性，腫瘍径の大きな腫瘍（>3 cm）の再発率は有意に高かった（p=0.0158，p=0.0162，p=0.0001）．Ki-67，c-erbB-2，p53 の過剰発現症例の再発率は，有意に高かったが（p=0.0035，p=0.0027，p=0.0076），MRP 発現と再発とは関連がなかった．多変量解析では，腫瘍径の大きな腫瘍と Ki-67 高発現が再発の独立した予後因子であった．腫瘍径が 1 cm 未満の症例の再発は，Ki-67 および p53 過剰発現が関連した．Ki-67 高発現により，grage 2，pT1 および単発腫瘍において，再発の高い症例を識別できた．c-erbB-2 過剰発現は，広基性，腫瘍径の大きな腫瘍，多発性，grade 1 腫瘍において，再発とつよい関連を示した．p53 過剰発現は，pTa 腫瘍において，より高い再発危険性を示した．これらの結果は，増殖関連蛋白を臨床病理因子に加えることにより，重要な予後を規定する情報となることを示しており，Ki-67 高発現が信頼しうる再発予後因子であった．1 つの因子だけではなく多くの因子を組み合わせることにより，さらに正確な腫瘍再発の予測が可能であった．

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