STAG2 Coupled With p53 Alteration Has a Significant Impact on Bladder Cancer Recurrence and Progression

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Research article

Keywords: STAG2; P53; Bladder Cancer; Prognosis

Posted Date: August 29th, 2019

DOI: https://doi.org/10.21203/rs.2.13677/v1

Abstract

Background:There is an unmet need for additional biomarkers for stratifying tumors based on their risk of recurrence and progression. Stromal antigen 2 (STAG2) and p53 are the most common mutations in bladder cancer and their association with the prognosis of bladder cancer remains unclear.Methods:Patients were divided into two cohorts according to stage and surgical procedure. The value of combined STAG2 expression (+/-) and P53 mutation (+/-) was evaluated for prediction of recurrence in 334 patients diagnosed by transurethral resection of bladder tumor (TURBT) and for prediction of survival in 144 patients who underwent radical cystectomy and pelvic lymphadenectomy(RCPLT).Results:We found that,in the 334 TURBT-treated patients, recurrence rate was significantly greater for STAG2(+) tumors than STAG2(-) tumors (73.5% vs. 55.0%, P=0.001). Of 144 RCPLT-treated patients, 127 (88.2%) were STAG2(+), of which 71 (55.9%) survived 5 years, compared to none of the 17 STAG2(-) patients. Patients with combined STAG2(+)/P53 mutation(+) tumors had the highest recurrence rate (83.5%) while patients with STAG2(-)/P53(-) tumors had the lowest recurrence rate (50%). Patients with STAG2(+)/P53(-) tumors achieved the highest 5-year survival rate (69.7%). Systemic chemotherapy did not improve prognosis of RCPLT-treated patients, but appeared to benefit STAG2(-) patients.Conclusions: Our results suggest that combined STAG2 expression and P53 status is a valuable biomarker for BCa prognosis, accurately predicting recurrence in TURBT-treated patients. STAG2(+)/P53(-) predicted a higher five-year survival and STAG2(-) predicted low five-year survival in RCPLT-treated patients. Chemotherapy may interfere with this predictive efficacy, especially in STAG2(-) patients.

Background

Bladder cancer (BCa) or urothelial cell carcinoma is the fourth most common neoplasm in Western males, and most are early-stage tumors known as papillary non–muscle-invasive bladder cancers (NMIBCs).[1,2] After resection, up to 70% of NMIBCs recur locally, and up to 20% of these recurrences progress to muscle invasion [3–5] and lethal metastatic bladder cancer, with only 5% of patients surviving 5 years.[6] Additional biomarkers are required for stratifying tumors based on risk of recurrence and progression.

Established BCa risk factors include exposure to industrial aromatic amines, cigarette smoke, and various other DNA-damaging agents[7–10] that can cause DNA mutation and functional protein loss.[11,12] A recent study found that STAG2 is one of the most commonly mutated genes in BCa.[13] The STAG2 protein is a component of the cohesin complex, which functions in diverse genomic processes such as chromosome segregation, regulation of chromatin structure, gene expression, and DNA repair.[14–16] Loss of STAG2 expression or STAG2 mutation is associated with BC prognosis,[17] but the net effects on recurrence and survival have differed across studies. One study reported that loss of STAG2 expression(STAG2 mutation) was associated with better prognosis of both NMIBC and MIBC,18 while another concluded that loss of STAG2 expression was associated with increased risk of disease recurrence and cancer-specific mortality. Recent studies have shown that STAG2 gene can predict the recurrence rate and progression of bladder cancer patients after TURBT. 19 However, it is not clear

whether STAG2 gene can predict the recurrence rate and progression of bladder cancer patients after other surgical procedures.

Inactivating mutations of STAG2 have recently been identified in human cancer and were demonstrated to cause chromosome segregation defects and aneuploidy.[20,21] As the STAG2 gene is on the X chromosome, complete genetic inactivation requires only a single mutational event in males,[22] leading to a significantly higher prevalence of BCa in males than females.[23] Further, male mice develop and die from N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN)-induced bladder cancer than frequently female mice.[24] This sex difference in BBN-induced BCa is unlikely due to differences in exposure and initial response to the carcinogen because the BBN-induced DNA mutation rates did not differ between sexes.[25] A more recent study reported that the X chromosome protects against BCa via upregulation of the canonical P53 pathway.[26] We and others found that p53 mutation has a significant impact on BCa outcome,[27–29] suggesting that both STAG2 and P53 mutations are associated with BCa recurrence and progression. Previous studies on the relationship between STAG2 and BCa prognosis have yielded discrepant results, possibly because they did not account for P53 gene mutations. To test this hypothesis, we conducted immunohistochemical analysis of STAG2 and P53 expression in 478 bladder tumors of different stages and grades. We also investigated the relationships among STAG2 expression, P53 expression, BCa recurrence, and BCa progression.

Methods

Patients and Tissue Specimens

The entire study population consisted of two cohorts divided according to the source of tumor tissue. Once patients were evaluated against exclusion criteria, the final cohort was composed of 478 patients. Exclusions included patients: (i) whose cut tumor block displayed poor integrity on the slide mount, (ii) whose cut slides had no identifiable tumor, (iii) whose tumor was carcinoma insitu (CIS), (iv) whose tumor was adenocarcinoma,(v) whose clinical information in the cancer registry was sufficiently ambiguous that the presence or absence of recurrence could not be determined,(vi) who received preoperative chemotherapy or radiotherapy,(vii) who underwent total resection received chemotherapy, but the chemotherapeutic drugs were not platinum and (or) gemcitabine.All of these exclusions were performed prior to data analysis.

The first cohort consisted of 334 patients diagnosed by transurethral resection of bladder tumor (TURBT) and the second of 144 patients who underwent RCPLT. The entire population of 478 patients included 303 males and 175 females receiving first BCa surgery and with no history of chemotherapy or radiotherapy before operation. Paraffin specimens were obtained mainly from Bethune International Peace Hospital, 324 Hospital, Third Military Medical University Southwest Hospital, and Jiangbei District People's Hospital of Chongqing. In all cases, pathological specimens were well preserved. All 334 patients who underwent TURBT were then treated with intravesical instillation of hydroxycamptothecin (HCPT) or epirubicin to prevent tumor recurrence, The bladder perfusion scheme is: HCPT or epirubicin 30–50mg is

mixed with 50 ml saline, and the bladder perfusion treatment is carried out. The perfusion time is about 40 minutes, once a week for 8 weeks, and 8 times later, it is changed to once a month for 8 months. The patients receiving RCPLT were divided into two subgroups: no post-surgical chemotherapy or radiotherapy (76 patients) and post-surgical platinum- and (or) gemcitabine treatment (68 patients).

Patients were followed-up using hospital records and by telephone interviews with patients or families. Recurrence was defined as a new tumor in the bladder after primary treatment regardless of their pathological type: bladder ultrasonography was performed monthly and cystoscopy was performed once every three months within twelve months. Bladder ultrasonography was performed every three months after a year, and cystoscopy was performed every six months. Patients who underwent RCPLT were examined by ultrasonography two months after operation and platinum and (or) gemcitabine chemotherapy was started if tumor metastasis was found. Only BCa-related deaths were considered for survival analysis. The study was conducted according to Chinese law and ethics board approval was obtained for gathering all study material and patient data. All patients provided informed consent before participation in the study. The study was approved by the Ethics Committee of Chongqing Medical University.

Immunohistochemistry

We used STAG2 monoclonal antibody (1:100, Santa Cruz Biotechnology) and mouse monoclonal anti-human p53 (DO–1) antibody (1:200, Santa Cruz Biotechnology). Immunostaining was performed in an automated immunostainer (Leica Bond-Max) following 30-min of heat-induced antigen retrieval at high pH using epitope retrieval buffer (Bond-Max). Primary antibody was applied for 30 min, and Bond-Max polymer was applied for 15 min. Diaminobenzidine was used as the chromogen, and samples were counterstained with hematoxylin. Levels of STAG2 and P53 staining were scored in a blinded fashion by two independent observers at two different institutions. Results were scored for transitional cell carcinoma by estimating the proportion of tumor cells with characteristic nuclear staining. An arbitrarily defined 15% cutoff was taken to classify the TCC data into categorical groups(positive versus negative)

Statistical Analysis

All statistical analyses were conducted using SPSS® 13.0. Statistical power analysis was conducted to estimate the required numbers in each cohort. Continuous variables are presented as mean (standard deviation) and categorical variables as count (percentage). Kaplan–Meier curves were constructed for survival analysis and results compared by the log rank test and Cox proportional hazards models. Statistical significance was set at P = 0.05 (two-tailed) for all tests.

Results

Association between STAG2 expression and bladder cancer recurrence

The relationship between STAG2 expression and recurrence of bladder cancer was analyzed in 334 patients who underwent TURBT (Table 1). Among these patients, 233 exhibited high STAG2 expression [>15% of cells, STAG2(+)] (Fig1 A and C)and 101 patients exhibited low STAG2 expression [<15% of cells, STAG2(-)](Fig1 E and G). Of the 233 STAG2(+) patients, 172 recurred (73.8%) compared to only 55 of the 101 STAG2(-) patients (54.5%) (P = 0.001 by chi square test). Univariate analysis revealed that high expression of STAG2 was positively correlated with recurrence of bladder cancer (Table 3, log rank test P = 0.000). Kaplan-Meier analysis also demonstrated higher recurrence rate in STAG2(+) patients than STAG2(-) patients (Fig. 2A, P = 0.001).

Association of combined high STAG2 expression and P53 mutation with bladder cancer recurrence

In light of the strong association between p53 mutation(high expression) (Fig 1B and Fig 1F)with bladder cancer occurrence, we also examined P53 expression in these paraffin-embedded bladder tumor tissues. First, we investigated the relationship between combined STAG2/P53 mutation status and bladder cancer recurrence in 334 patients who underwent TURBT. Recurrence rates in the four combined groups were 83.5% for STAG2(+)/P53(+), 62.8% for STAG2(+)/P53(-), 72.7% for STAG2(-)/P53(+), and 50% for STAG2(-)/P53(-) (Table 2). We performed a univariate analysis to evaluate whether combined high STAG2/P53 staining was significantly associated with recurrence. Kaplan-Meier curves showed that STAG2(+)/P53(+) status more accurately predicted recurrence of bladder cancer than other combinations (Fig. 3A, P = 0.000). In STAG2(+) positive patients, P53 mutation promoted recurrence of bladder cancer (Fig 3C, P = 0.000). In STAG2(-) patients, P53 mutation also affected the recurrence of bladder cancer, but the difference between STAG2(-)/P53(+) and STAG2(-)/P53(-) groups did not reach significance (Fig. 3E, P = 0.077). To identify additional factors influencing recurrence of bladder cancer, we conducted multivariate analysis using the Cox proportional hazard model. Results revealed that STAG2 expression, P53 expression, and tumor grade had significant predictive value for recurrence (Table 4).

Correlation between STAG2 expression and bladder cancer progression

The relationship between STAG2 expression and 5-year survival rate of bladder cancer was analyzed in the second cohort of 144 patients who underwent RCPLT. Of these patients, 127 were STAG2(+) (88.2%). Five-year survival rate was higher among STAG2(+) than STAG2(-) cases (71/127 [55.9%] vs. 0/17 [0%], P<0.01 by chi square test) (Table 2). We then performed a univariate analysis to evaluate whether STAG2 staining was significantly associated with survival and found that STAG2(-) patients exhibited lower 5-year survival rate than STAG2(+) patients (Table 5, P = 0.000). Kaplan-Meier analysis also demonstrated significantly higher mortality in STAG2(-) patients than STAG2(+) patients (Fig. 2B, P = 0.001).

Association of combined STAG2 expression and P53 mutation with bladder cancer progression

The relationship between combined STAG2(+)/P53 mutation status and 5-year survival rate of bladder cancer was analyzed in the second cohort. Of 127 STAG2(+) patients, 5-year survival was significantly lower in those with p53 mutation (+) compared to those with normal P53 expression (41% vs. 69.7%, P<0.05 chi square test)(Table 1). Thus, P53 mutation may promotes bladder cancer progression. We also

performed a univariate analysis to evaluate whether combined STAG2(+)/P53(+) status was predictive of survival(Table 5). Indeed, the Kaplan-Meier curve showed that combined status predicted survival more accurately than individual STAG2 or P53 status (Fig. 3B, P = 0.00). In STAG2(+) patients, P53 mutation reduces survival rate compared to P53(-) patients (Fig. 3D, P = 0.01). In STAG2(-) patients, the effect of P53 mutation on prognosis was even stronger (Fig. 3F, P = 0.001). To further study the factors affecting bladder cancer survival, multivariate analysis of the Cox proportional hazard model was conducted and revealed that STAG2 and P53 expression had significant clinical predictive value for cancer-specific survival (Table 6)

Affects of chemotherapy on bladder cancer progression

Chemotherapy is one of the main treatments for bladder cancer. After TURBT, all patients were treated with intravesical chemotherapy, so the chemical treatment effect was difficult to assess among STAG2/P53 expression subgroups. We thus studied the effect of chemotherapy on the prognosis of patients with invasive bladder cancer in the second cohort of 144 patients. Systemic chemotherapy did not improve prognosis, as 5-year survival rate did not differ significantly between treatment subgroups (Table 6). Since the five-year survival rate of STAG2(-) patients was 0%, we compared the 3-year survival rate and found greater survival among the chemotherapy subgroup than the no chemotherapy subgroup (5/11 [45.4%] vs. 1/6 [16.7%]). We thus speculate that systemic chemotherapy may prolong the survival of RCPLT-treated STAG2(-) patients. To test this hypothesis, we performed a univariate analysis to evaluate whether chemotherapy was significantly associated with survival. Chemotherapy did not reduce overall mortality (Fig. 4A, P = 0.292) or significantly improve the prognosis of STAG2(+) patients (Fig. 4C and Fig. 4D, P>0.05), but may have modest effects for STAG2(-).

Discussion

Bladder cancer is a heterogeneous epithelial malignancy that presents most commonly as an exophytic tumor confined to the mucosa or lamina propria.[30] The recurrence and invasiveness of bladder cancer are associated with poor prognosis. Thus, additional biomarkers are required assessment of recurrence risk, progression, and treatment response.

Recent studies indicate that STAG2 mutation is strongly associated with prognosis.[17–19] However, some studies have reached the opposite conclusion. In the current study, high expression of STAG2 was closely related to recurrence and poor prognosis in NMIBC patients. In MIBC patients, however, low STAG2 expression promoted bladder cancer progression. In our study, we also found similar conclusions. The biological basis for the different effects of STAG2 expression on the clinical outcomes of non-muscle-invasive papillary carcinomas versus muscle-invasive carcinomas is currently unknown.

To further examine the relationship between STAG2 and bladder cancer prognosis, we analyzed other prognostic factors, including expression of the tumor suppressor p53. Indeed, P53 gene mutation(the mutant P53 gene was highly expressed after immunohistochemical staining)significantly affected

prognosis of STAG2(+) patients. Recurrences was higher in STAG2(+)/P53(+) patients than STAG2(+)/P53(-) patients (84.5% vs. 58%), indicating that P53 mutation is a major cause of BCa recurrence.[31,32] All NMIBC patients were treated with intravesical chemotherapy after operation, and P53 mutation may reduce chemosensitivity.[33] Similarly, previous studies have shown that STAG2 is an important gene for homologous recombination repair.[34] STAG2 overexpression may increase chemotherapy resistance by enhancing tumor cell DNA damage repair capacity. We found that both STAG2 mutation [STAG2(-)] and P53 mutation [P53(+)] promoted progression in MIBC patients who underwent RCPLT. Indeed, no STAG2(-)/P53(+) patient in our study survived for 5 years, while 5-year survival was relatively high in STAG2(+)/P53(-) patients (69.7%). This suggests that like P53, STAG2 may be a suppressor of tumor progression. But why do STAG2(+) BCa patients relapse more easily after TURBT? Poor sensitivity to chemotherapy in patients with STAG2 (+) may be one of the causes of its recurrence.

Chemotherapy may improves tumor prognosis and many chemotherapeutic drugs induce tumor cell apoptosis by damaging DNA.[35,36] The sensitivity of tumor cells to chemotherapeutic drugs influences tumor prognosis.[37,38] In this study, we found that systemic chemotherapy did not improve the 5-year survival rate of MIBC patients. Alternatively, STAG2(-) patients receiving chemotherapy demonstrated higher 3-year survival than those not receiving chemotherapy (45.5% vs. 16.7%). Alternatively, chemotherapy provided no survival benefit to STAG2(+) patients. Furthermore, univariate analysis suggested that STAG2(-) patients were more sensitive to chemotherapy than STAG2(+) patients, but it was difficult to draw conclusions because of the insufficient sample size. From these results, we speculate that chemotherapy (intravesical instillation chemotherapy) reduces relapse of STAG2(-) NMIBC due to a superior effect of intravesical instillation chemotherapy versus systemic chemotherapy for bladder cancer.[39]

Cancer prognosis is strongly related to the genotype of tumor cells and specifically how different genotypes and expression phenotypes affect growth, progression, and treatment response.[40,41] In this study, we also compared the prognosis of MIBC patients with different expression phenotypes after chemotherapy. STAG2(+)/P53(-) patients still had better prognosis than STAG2(-)/P53(+) patients even after chemotherapy, possibly due to sustaining progression of mutant tumor cells and (or) inefficacy of chemotherapy. In bladder cancer, mutations in the P53 gene and STAG2 gene lead to tumor progression, and systemic chemotherapy cannot effectively inhibit this process, resulting in poor prognosis in STAG2(-)/P53(+) patients.

Our previous colleagues found that total resection of early bladder cancer significantly reduced recurrence and mortality. In clinical treatment, patients are willing to choose total cystectomy and bladder substitution after knowing that the diagnosis of bladder cancer is bladder cancer, which leads to partial bladder surgery for early stage tumors. We will follow up these patients for a long time to see if their long-term survival rate has improved. Preoperative chemotherapy may cause changes in STAG2 or P53 expression in surgical specimens. We excluded patients who received preoperative chemotherapy or

radiotherapy. After total cystectomy, some patients received cisplatin combined with gemcitabine systemic chemotherapy (other drug chemotherapy patients were excluded), and some patients did not undergo systemic chemotherapy because of the low stage of the tumor during the operation.

However, this does not affect our purpose. We focus on identifying the factors that influence the relationship between STAG2 and the prognosis of bladder cancer. STAG2 is a functional gene on X chromosome. Recently, it has been found that X chromosome can regulate P53 pathway. Therefore, we first speculate that STAG2 may affect the prognosis of bladder cancer through its association with P53. In addition, the most important way to affect the prognosis of bladder cancer is clinical treatment and chemotherapy. Therefore, the focus of our study is to analyze whether these factors are related to STAG2.

We tested different thresholds used to define positivity or loss of expression of STAG2. We found that defined 15% as the cutoff frequency for STAG2 and P53 to classify transitional cell carcinoma data into categorical groups is the most reasonable result.

Conclusions

we confirm the association between combined STAG2/P53 status and bladder cancer prognosis. Indeed, combined STAG2 expression/P53 mutation status can more accurately predict the prognosis of bladder cancer, whether NMIBC or MIBC and with or without postoperative chemotherapy, compared to either marker alone. For bladder cancer prognosis by STAG2 and P53, only the surgical approach (tumor stage) need be considered, regardless of the degree of tumor differentiation. If the method is effectively promoted, it will be beneficial for individualized treatment and medical decision making.

Abbreviations

BCa:Bladder cancer

STAG2:Stromal antigen 2

RCPLT: Radical cystectomy and pelvic lymphadenectomy

NMIBCs:Non–muscle-invasive bladder cancers

BBN:N-butyl-N-(4-hydroxybutyl)nitrosamine

TURBT:transurethral resection of bladder tumor

Declarations

Acknowledgments

The authors would like to thank Qing Zhou (Department of Pathology) and Dr. Junlei Zhang (Department of Histology and Embryology) and Jie An (Department of Pathology , Bethune International Peace Hospital) for their excellent technical assistance and their critical reading and valuable comments on the manuscript.

Funding

This work was supported by Science and Technology Commission Fund of Yongchuan District, Chongqing, China (Ysctc,2017nc5009), and the Research Foundation of Yongchuan Hospital of Chongqing Medical University (YJYI201604,YJSCI201932).

Ethics approval and consent to participate

All pa¬tients provided informed consent before participation in the study. The study was approved by the Ethics Committee of Chongqing Medical University.

Availability of data and materials

The basic patient information and IHC staining results have been shown inTable 1,Table 2 and Table 5.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Jiangchuan Chen, Zhigang Xu and Xiao-Dong Meng for acquisition of data, analysis and interpretation of data,statistical analysis and drafting of the manuscript. Yin Chen and Jie Li for technical and material support. Jie Li for study concept and design ,analysis and interpretation of data, drafting of the manuscript, obtained funding and study supervision. All authors read and approved the final manuscript.

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Tables

Table 1 Decreased STAG2 expression in human bladder cancer

	STAG2(+)	STAG2(-)	Total	P Value
Gender:				
Men	269	94	363	0.791
Women	91	24	115	
Age:				
60 or less	132	41	173	0.706
Greater than 60	228	77	305	
Pathological grade:				0.000
PUNLMP	92	21	113	
High grade	141	29	170	
Low grade	127	68	195	
Pathological stage:				0.000
Ta-T1	233	101	334	
T2-T4	127	17	144	
Recurrence or not				0.001
Recurrence	172	55	227	
Not recurrence	62	45	107	
Survival or not				0.001
survival	71	0	71	
Dead	56	17	73	
p53 status:				0.000
P53(+)	182	25	207	
P53(-)	179	92	271	

Table 2 Association of STAG2 and P53 expression with tumor differentiation and prognosis

5y recurrence rate in first trial	Recurrence	Not recurrence	Total tumors	P Value
STAG2(+)	172	62	234	P=0.001
STAG2(-)	55	45	100	
STAG2(+)/P53(+)	101	20	121	P=0.000
STAG2(+)/P53(-)	71	42	113	Pa=0.000
STAG2(-)/P53(+)	16	6	22	Pb=0.058
STAG2(-)/P53(-)	39	39	78	Pc=0.000
5y survival rate in second trial	**Survival**	**Dead**	**Total tumors**	**P Value**
STAG2(+)	71	56	127	P=0.001
STAG2(-)	0	17	17	
STAG2(+)/P53(+)	25	36	61	P=0.000
STAG2(+)/P53(-)	46	20	66	Pa=0.001
STAG2(-)/P53(+)	0	3	3	Pb=0.386
STAG2(-)/P53(-)	0	14	14	Pc=0.012

Note: P is a comparison between the whole groups.Pa is a comparison between STAG2(+)/P53(+) group and STAG2(+)/P53(-) group. Pb is a comparison between STAG2(-)/P53(+) group and STAG2(-)/P53(-) group;Pc is a comparison between STAG2(+)/P53(+) group and STAG2(-)/P53(-) group.

Table 3 Univariate analysis of clinical characteristic influence on recurrence in 334 patients with bladder cancer

Parameter	No.patients	P Value (log rank test)	Hazard ratio	Lower CL	Upper CL
Gender:		0.922	0.976	0.606	1.573
Women	83				
Men	251				
Age:		0.792	1.067	0.661	1.722
60 or less	122				
Greater than 60	212				
Pathological grade:		0.005	0.675	0.512	0.891
PUNLMP	123				
Low grade papillary Ca	95				
High grade papillary Ca	126				
STAG2:		0.000	2.358	1.447	3.844
Positive	233				
Negative	101				
P53:		0.000	0.287	0.171	0.481
Positive	143				
Negative	191				
STAG2 and P53		0.000	1.596	1.305	1.954
STAG2(+)P53 (+)	121				
STAG2(+)P53(-)	113				
STAG2(-)P53(+)	22				
STAG2(-)P53(-)	78				

Table 4 Cox multivariate regression analysis of potential recurrence predictive facors in 334 patients with bladder cancer

Variable	Category	RR(95% CI)	P Value
Age	Less than 60/60 or greater	1.001(0.763-1.313)	0.995
Grender	Men/Women	1.039 (0.792-1.364)	0.782
Grade	High/Low grade papillary Ca/PUNLMP	0.578(0.418-0.799)	0.001
STAG2	(-)/(+)	0.576 (0 .413-0.804)	0.001
P53	(-)/(+)	0 .643 (0 .486-0.851)	0.002

Table 5 Univariate analysis of clinical characteristic influence on survival in 144 patients with bladder cancer

Parameter	No.patients	P Value (log rank test)	Hazard ratio	Lower CL	Upper CL
Gender:		0.825	0.860	0.392	1.884
Women	32				
Men	112				
Age:		0.360	0.586	0.268	1.275
60 or less	51				
Greater than 60	93				
Pathological grade:		0.019	2.103	0.994	4.449
Low grade papillary Ca	75				
High grade papillary Ca	69				
STAG2:		0.000	0.000	0.000	
Positive	127				
Negative	17				
P53:		0.092	3.103	1.456	6.614
Positive	67				
Negative	77				
STAG2 and P53		0.000	0.000	0.000	1.954
STAG2(+)P5(3+)	61				
STAG2(+)P53(-)	66				
STAG2(-)P53(+)	3				
STAG2(-)P53(-)	14				
Chemotherapy		0.399	0.989	0.461	2.123
Chemotherapy	68				
Not chemotherapy	76				

Table 6 Cox multivariate regression analysis of potential survival predictive facors in 144 patients with bladder cancer

Variable	Category	RR(95% CI)	P Value
Age	Less than 60/60 or greater	1.317(0.820-2.115)	0.255
Grender	Men/Women	1.145(0.700-1.873)	0.590
Grade	High/Low grade papillary Ca/PUNLMP	0.693(0.427-1.124)	0.138
Chemotherapy	Chemotherapy/not	1.000(0.616-1.623)	0.999
STAG2	(-)/(+)	6.265 (3 .103-12.651)	0.000
P53	(-)/(+)	0 .380 (0 .220-0.659)	0.001

Figures

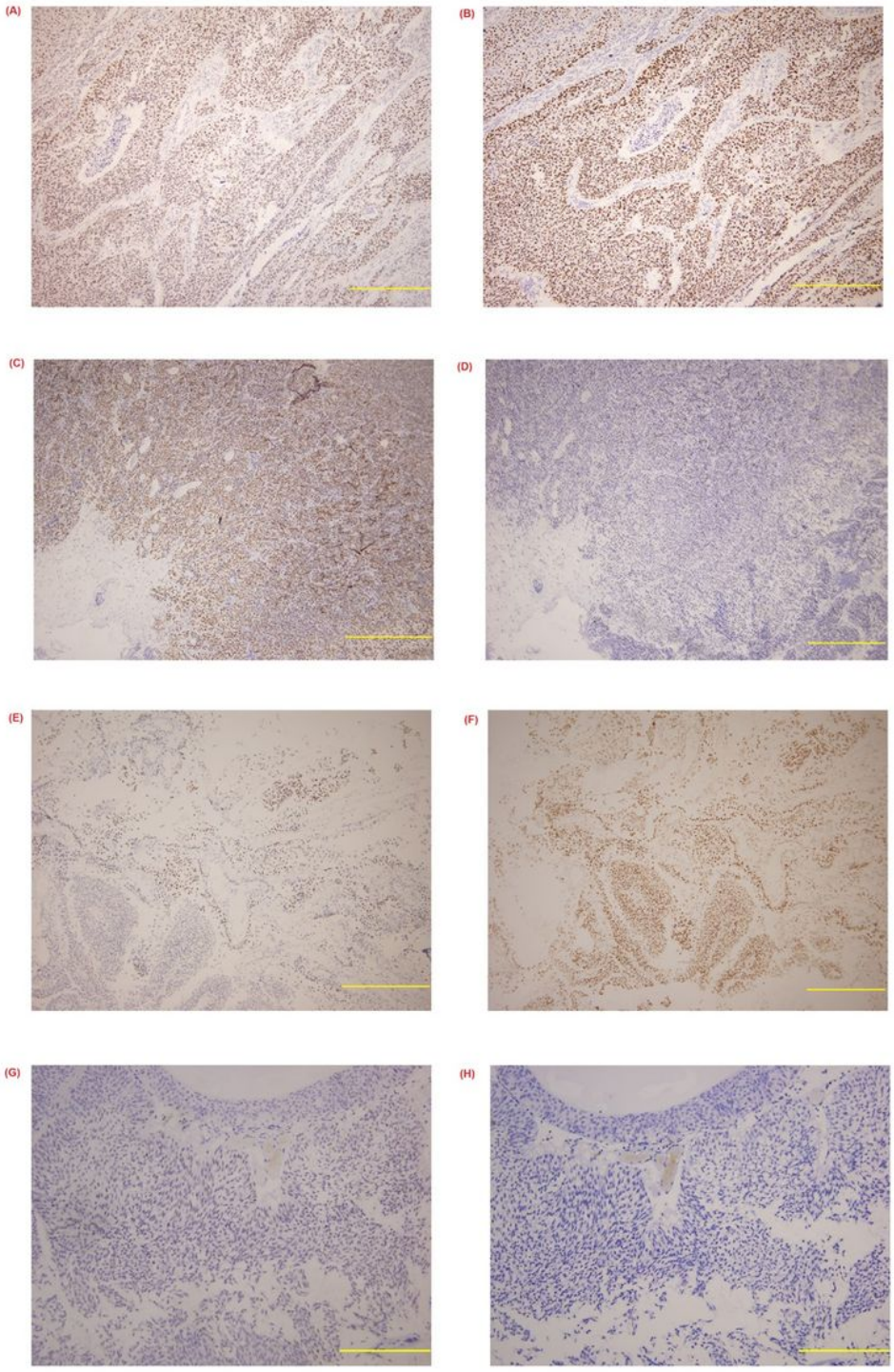

Figure 1

Immunohistochemical staining of paraffin embedded bladder urothelial carcinoma. (A and B) Positive STAG2 expression (no mutation) and positive P53 expression (P53 mutation). Immunohistochemistry analysis can help determine the p53 mutation because a nuclear localization of p53 represents p53 mutations, which is a widely accepted consensus in most p53 studies.(C and D) Positive STAG2

expression and negative P53 expression42. (E and F) Negative STAG2 expression (STAG2 mutation) and positive P53 expression. (G and H) Negative STAG2 expression and negative P53 expression.

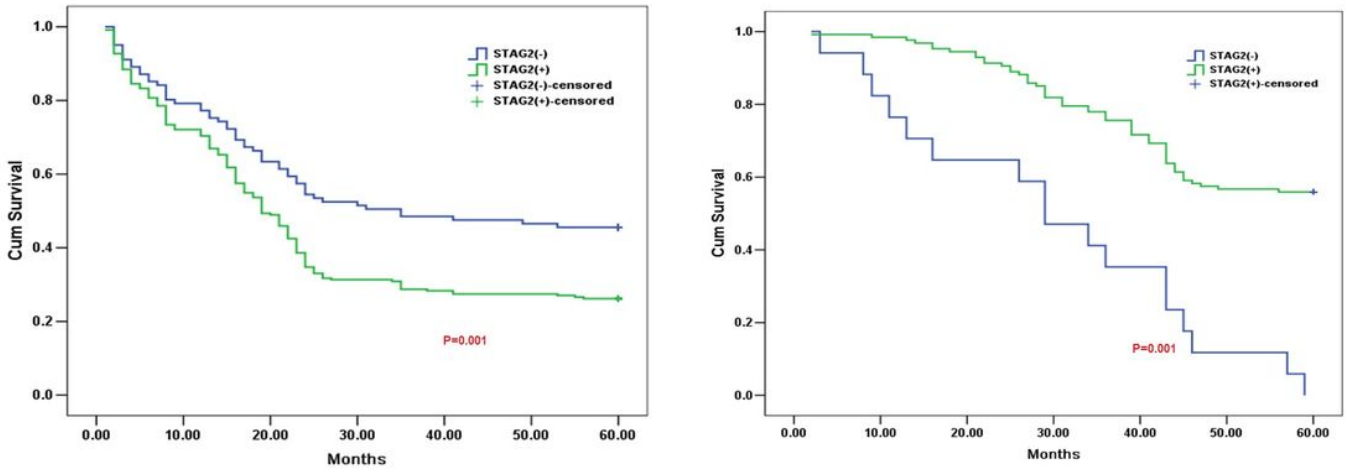

Figure 2

Kaplan-Meier plots showing the association of STAG2 expression with bladder cancer outcome. (A) Recurrence of bladder cancer in TURBT-treated STAG2(+) patients (n=234) versus STAG2(-) patients (n=100). (B) Cancer-specific survival of RCPLT-treated STAG2(+) patients (n = 127) versus STAG2(-) patients (n = 17). P values correspond to results from multivariable analysis.

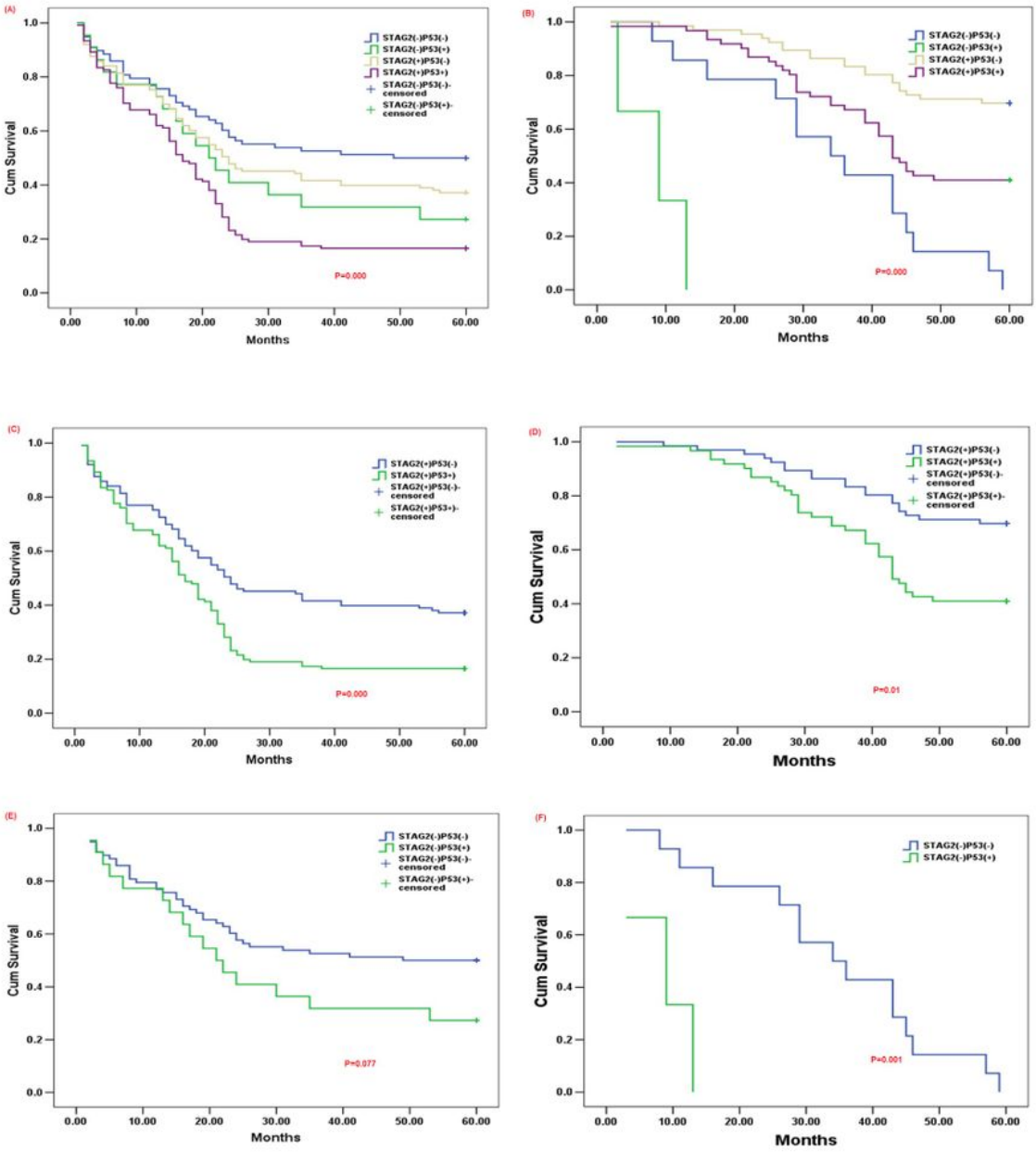

Figure 3

Kaplan-Meier curves showing the association of combined STAG2 expression and P53 status with bladder cancer outcome. (A) Recurrence of bladder cancer among TURBT-treated patients of the four STAG2/P53 phenotypes: STAG2(+)/P53(+), STAG2(+)/P53(-), STAG2(-)/P53(+), and STAG2(-)/P53(-). (B) Cancer-specific survival among RCPLT-treated patients according to STAG2/P53 phenotype. (C) Comparison of bladder cancer recurrence between TURBT-treated STAG2(+)/P53(+) and STAG2(+)/P53(-)

patients. (D) Comparison of cancer-specific survival between RCPLT-treated STAG2(+)/P53(+) and STAG2(+)/P53(-) patients. (E) Comparison of bladder cancer recurrence between TURBT-treated STAG2(-)/P53(+) and STAG2(-)/P53(-) patients. (F) Comparison of cancer-specific survival between RCPLT-treated STAG2(-)/P53(+) and STAG2(-)/P53(-) patients. P values correspond to results from multivariable analysis.

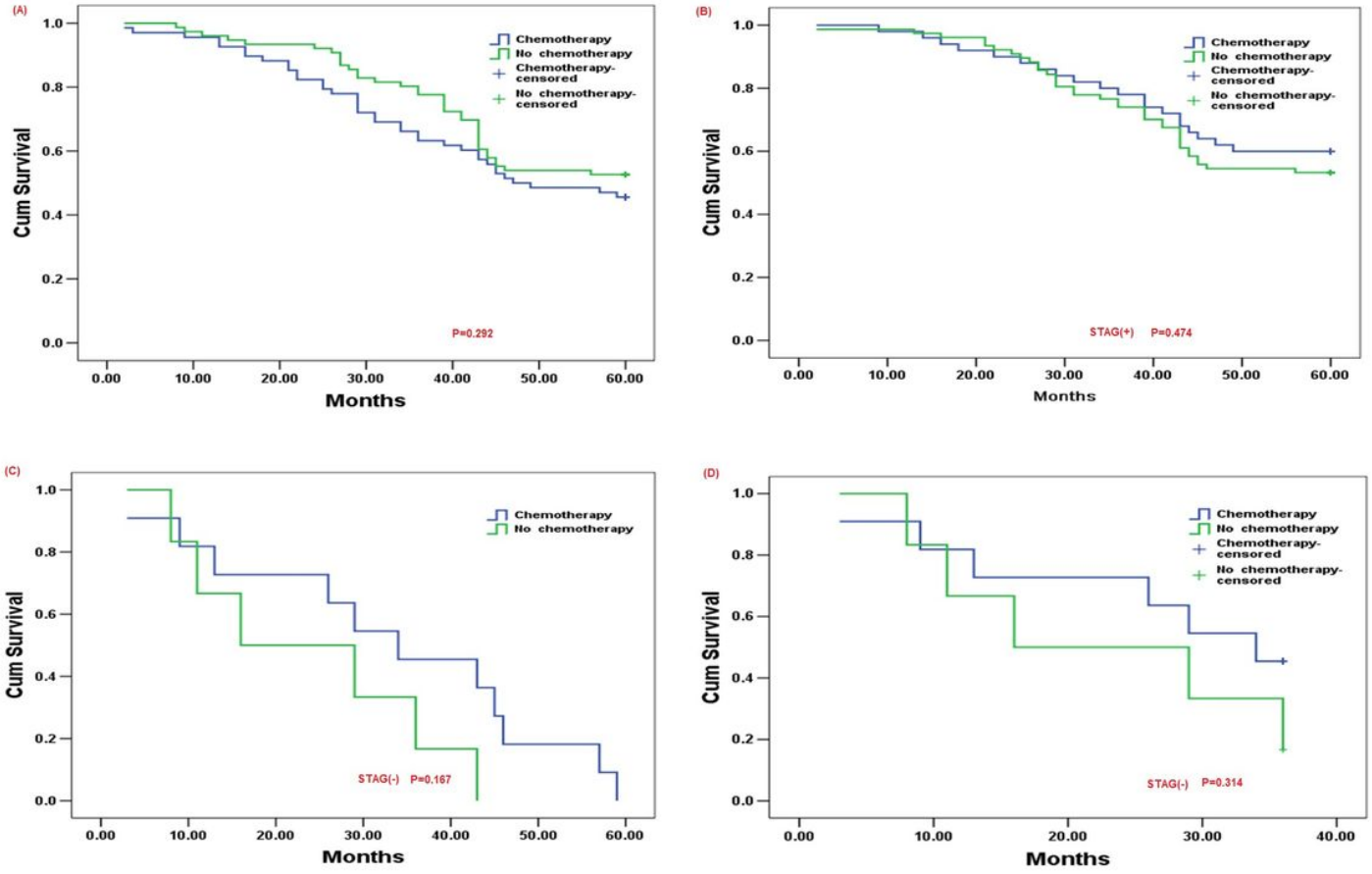

Figure 4

Kaplan-Meier plots of the association between chemotherapy and outcome in RCPLT-treated bladder cancer patients (A) Five-year cancer-specific survival of RCPLT-treated patients receiving or not receiving subsequent chemotherapy. (B) Five-year cancer-specific survival of RCPLT-treated STAG2(+) patients with and without chemotherapy. (C) Five-year cancer-specific survival of RCPLT-treated STAG2(-) patients with and without chemotherapy. (D) Three-year cancer-specific survival of RCPLT-treated STAG2(-) patients with and without chemotherapy. P values correspond to results from multivariable analysis.