Int J Clin Exp Pathol 2015;8(5):4999-5007
www.ijcep.com /ISSN:1936-2625/IJCEP0006923

Original Article

Clinicopathologic and prognostic significance of p21 (Cip1/Waf1) expression in bladder cancer

Kun Tang[1,2*], Chenghe Wang[1,2*], Zhong Chen[1,2], Hua Xu[1,2], Zhangqun Ye[1,2]

[1]Department of Urology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China; [2]Institute of Urology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China. [*]Equal contributors.

Received February 9, 2015; Accepted April 12, 2015; Epub May 1, 2015; Published May 15, 2015

Abstract: Recent studies have shown that altered expression p21 is shown to associate with tumorigenesis and tumor progression. To investigate the clinicopathological significance and prognostic value of p21 in bladder cancer (BCa). A total of 48 patients with BCa were included in this study. The correlation between p21 expression and clinicopathologic features and survival was studied. Also, a meta-analysis was performed to investigate the relationship between the p21 and BCa survival. Low p21 expression was detected both in tumor tissues compared with adjacent normal tissues. The expression of p21 was closely associated with advanced pathologic TNM stage (P = 0.001) and tumor grade (P = 0.013). Moreover, patients with low p21 expression had shorter recurrence-free survival (P = 0.016) and overall survival rates (P = 0.039). Multivariate Cox regression analysis revealed that p21 low expression was an independent prognostic factor for recurrence free survival (P = 0.03). Additionally, our meta-analysis. The available outcome data from six articles were examined. A meta-analysis of the HR indicated a significantly poor overall survival (OS, HR: 1.75, 95% CI: 1.38-2.21), recurrence free survival (RFS, HR: 1.83, 95% CI: 1.57-2.15), progression free survival (PFS, HR: 2.02, 95% CI: 1.48-2.75), and cancer specific survival (CSS, HR: 1.89, 95% CI: 1.53-2.33) in patients with low expression levels of p21. Our present results indicated that low p21 expression predicated tumor recurrence and poor prognosis in bladder cancer.

Keywords: p21 (Cip1/Waf1), recurrence, prognosis, bladder cancer

Introduction

Bladder cancer is the fourth most common cancer worldwide [1] and is the most common genitourinary malignancy in China [2]. Contemporarily, radical cystectomy with pelvic lymphadenectomy and urinary diversion remains the mainstay of treatment for muscle-invasive diseases, while transurethral resection of the bladder in combination with intravesical chemotherapy or immunotherapy is considered as the standard treatment for nonmuscle-invasive bladder cancer (NMIBC) [3, 4]. Several clinicopathological factors, such as tumor-node-metastasis (TNM), pathological stage and grade of the tumor, tumor multiplicity, tumor size are demonstrated to predict prognosis of BCa. So far, effective predictive tools or nomograms are absent for bladder cancer after surgical procedures, thus more studies are needed to better evaluate the existing predictors and discover the promising ones. Therefore, identification of BCa specific disease-related biomarkers that can predict the clinical outcome is of great importance and urgently needed.

Using immunohistochemistry, recent studies has revealed that p21 was expressed in a variety of human malignancies, and is correlated with tumor progression and a poor prognosis in various carcinomas [5, 6]. Altered tumor suppressor gene p21 has been reported to be associated with BCa progression. Our previous studies have shown that dsRNAs transfection [7] and miRNAs complementary to p21 promoter [8] both have anti-tumor activity by activating p21 expression in BCa cell lines.

Although evidence exists that p21 is an important factor implicated in clinicopathological features and predicated the prognosis of BCa [9-21], however, as a matter of contradictory results as well as the small sample size in soli-

tary study, the prognostic significance of p21 expression for survival in patients with BCa still remains controversial. A study on p21 in BCa reported that positive p21 expression tended to indicate worse survival outcomes, but the difference was not statistically significant [27]. Moreover, two other recent studies on a panel of tumor markers demonstrated that p21 expression was not an independent prognostic factor for patients with BCa [12]. Whether discrepancy in these data was due to limited sample sizes or genuine heterogeneity is still unknown. To address this controversial issue, first, a cohort of a carried out to validate the association between p21 and clinicopathological parameters as well as the significance of p21 expression in the prediction of clinical outcomes in BCa, then a meta-analysis was performed to investigate the prognostic value of p21 in BCa.

Materials and methods

Sample collection

Forty-eight BCa and adjacent normal tissue samples (collected post-operatively from Nov 2010 to Feb 2014) from cystectomy were acquired in this study from the Department of Urology in Tongji Hospital of Huazhong University of Science and Technology (Wuhan, China). All the diagnoses were based on pathological history. The tissues were obtained before chemotherapy and radiation therapy. Upon removal of the surgical specimen, each sample was immediately frozen in liquid nitrogen and tored at -80°C prior to RNA isolation and qRT-PCR analysis.

RNA extraction and qRT-PCR

Total RNA was isolated from the frozen tissue sample with TRIzol (Invitrogen) according to the manufacturer's instructions. First-strand complementary DNA (cDNA) was synthesised from 2 μg of the total RNA using an oligo-dT primer and superscript II reverse transcriptase (Invitrogen). Then, quantification of the most up-regulated or down-regulated miRNAs was performed by qRT-PCR using SYBR Premix Ex Taq on MX3000 instrument. The U6 primers were obtained from GeneCopoeia. PCR was performed in a real-time PCR system as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 10 sec, 60°C for 20 sec and 72°C for 30 sec, and then 95°C for 1 min and 60°C for 1 min. All experiments were done in triplicate. The expression level values were normalized to those of the small nuclear RNA U6 as a control. Relative fold-changes of miRNA expression were calculated using the ΔΔCT method, and the values were expressed as $2^{-\Delta\Delta CT}$.

Immunohistochemistry

5 mm-thick paraffin-embedded tissue sections were prepared and then deparaffinized in xylene and rehydrated in series of ethanol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min at room temperature. Antigen retrieval was executed in 0.01 M sodium citrate buffer (pH 6.0) at 95°C for 45 min. After incubated in 5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 10 min, the tissue sections were incubated with the primary anti-p21 antibody (Cell Signaling Technology, USA) at a dilution of 1:300 overnight at 48°C. The sections were incubated with the secondary antibody for 20 min at room temperature and subjected to incubation with Envision Dual Labeled Polymer kit (BioGenex, San Ramon, CA) according to the manufacturer's instructions. The sections were then counterstained with hematoxylin. Tissue sections of lung squamous carcinoma were used as positive controls and tissue sections incubated with PBS to substitute the primary antibody were used as negative controls.

Western blot analysis

Whole-cell lysates, cytoplasmic and nuclear fractions were prepared. Protein were separated by 10% SDS/PAGE and transferred onto PVDF membranes (Millipore, USA). Membranes were incubated with appropriate antibodies for 1 h at room temperature or overnight at 4C followed by incubation with a secondary antibody. The following antibodies were used: anti-p21 (Cell Signaling Technology, USA) at 1:1000 dilution; anti-GAPDH, anti-Lamin B (Boster, China) at 1:500 dilution.

Follow-up

The length of follow-up was calculated from the date of surgery to the date of event or last clinical follow-up. The spectrum of clinical follow-up included a history, physical examination, and routine biochemical profile.

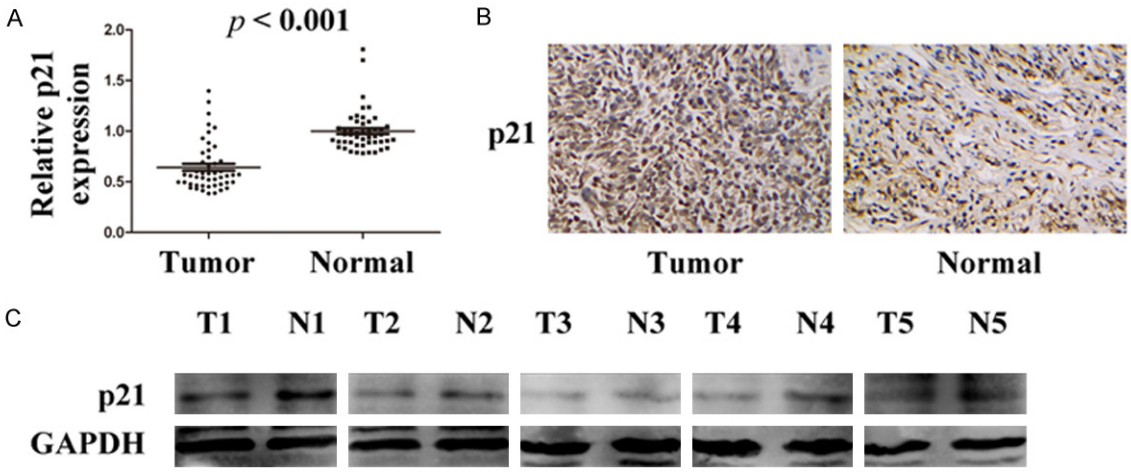

Figure 1. p21 expression is down-regulated in bladder cancer. qRT-PCR (A), immunohistochemistry (B) and Western-blot (C) detected low p21 expression in bladder cancer compared with adjacent normal tissues.

Table 1. Associations between p21 expression, patient and tumor characteristics (n=48)

Parameter	No. of case	p21 expression	*p*-value
Age (years)			
> 65	29	0.63±0.28	0.56
< 65	19	0.67±0.21	
Gender			
Male	28	0.65±0.22	0.94
Female	20	0.64±0.28	
Tumor diameter (cm)			
< 3	26	0.69±0.47	0.11
> 3	22	0.58±0.48	
Tumor multiplicity			
Single	33	0.68±0.26	0.093
Multiple	15	0.56±0.17	
Stage			0.001
Ta	21	0.77±0.27	
T1	19	0.57±0.15	
T2-4	8	0.48±0.11	
Grade			0.013
G1	32	0.70±0.27	
G2/G3	16	0.52±0.10	

Survival analysis

For survival analysis, we used the Kaplan-Meier method to analysis the correlation between variables and cancer-specific survival, and the log-rank test to compare survival curves. We used the Cox regression model to do the univariable and multivariable survival analysis. Kaplan-Meier survival analysis was used to analysis the association between postoperative RFS, OS and the p21 expression level measured by qRT-PCR, and the resulting curves were divided into two classes (high and low expression in comparison to the mean level of p21 expression as the threshold). We selected the median value of p21 relative expression as the optimum cut-off score.

Meta-analysis

Hazard ratios (HRs) and 95% confidence intervals (CIs) were used to estimate the impact of p21 expression on RFS, OS, CSS and PFS. A combined HR > 1 implied a worse survival, and it was considered statistically significant if 95% CI for the combined HR did not overlap 1. We performed the meta-analysis by using the Review Manager Software (RevMan 5.1, Cochrane Collaboration, Oxford, UK).

Results

p21 expression is down-regulated in bladder cancer

The expression of p21 in 48 cases of BCa tissues and adjacent normal tissues was examined by qRT-PCR (**Figure 1A**), immunohisto-

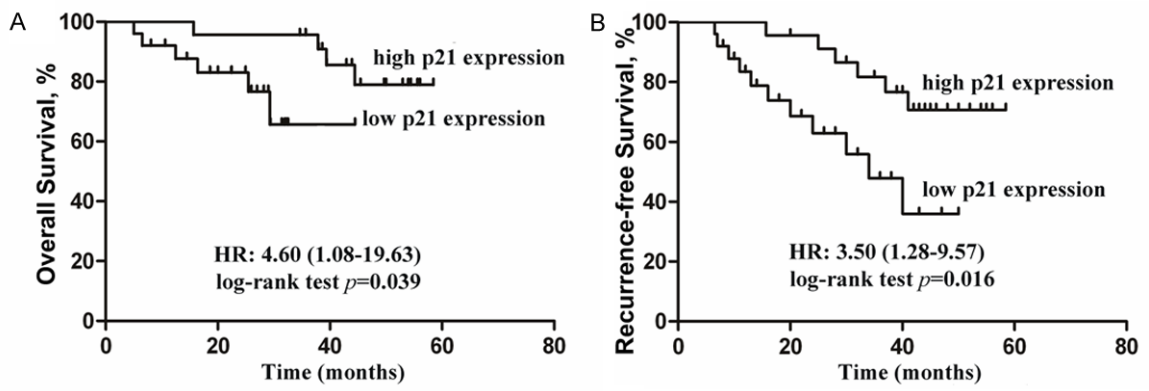

Figure 2. Kaplan-Meier estimates of overall survival (A) and recurrence free survival (B) in the cohort patients with bladder cancer.

Table 2. Univariate and multivariate analysis of the recurrence free survival and overall survival according to clinicopathological factors and p21 expression in all patients with bladder cancer (n=48)

Variables (and stratification)	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
RFS				
Age (> 65 years vs. < 65 years)	1.13 (0.57-2.25)	0.73	/	/
Sex (male vs. female)	1.08 (0.58-2.02)	0.81	/	/
Tumor size (> 3 cm vs. < 3cm)	2.76 (0.70-10.84)	0.15	/	/
Tumor multiplicity (multiple vs. single)	1.92 (0.90-4.11)	0.09	/	/
Stage (T2-4 vs. Ta-1)	5.36 (2.12-13.56)	< 0.001	5.01 (1.57-15.98)	0.007
Grade (G2-3 vs. G1)	5.01 (1.98-12.68)	< 0.001	3.45 (1.36-8.73)	0.009
p21 expression (low *vs.* high)	3.50 (1.28-9.57)	0.01	3.06 (1.13-8.26)	0.03
OS				
Age (> 65 years vs. < 65 years)	1.20 (0.68-2.10)	0.53	/	/
Sex (male vs. female)	1.05 (0.47-2.31)	0.91	/	/
Tumor size (> 3 cm vs. < 3cm)	2.63 (0.69-10.01)	0.16	/	/
Tumor multiplicity (multiple vs. single)	1.66 (0.77-3.54)	0.19	/	/
Stage (T2-4 vs. Ta-1)	7.47 (1.67-33.38)	0.008	4.84 (1.24-18.85)	0.02
Grade (G2-3 vs. G1)	10.18 (2.62-39.61)	< 0.001	9.52 (1.60-56.52)	0.01
p21 expression (low *vs.* high)	4.60 (1.08-19.63)	0.04	4.08 (0.88-18.97)	0.07

RFS, recurrence free survival; OS, overall survival; HR, hazard ratio; 95% CI, 95% confidence interval.

chemical analysis (**Figure 1B**), and western blot analysis (**Figure 1C**). A significant difference was observed in p21 expression between BCa and normal samples ($P < 0.05$).

Associations between p21 expression, patient and tumor characteristics

Next, we associated p21 expression with clinicopathological data from the patients (**Table 1**) and found that p21 expression was associated with tumor grade and stage ($P = 0.013$ and $P = 0.001$, respectively), but not associated with other data, such as age ($P = 0.72$), gender ($P = 0.49$), tumor diameter ($P = 0.21$), or tumor multiplicity ($P = 0.12$).

Association between p21 expression and survival in the full cohort

The association between p21 expression and patient survival was investigated using Kaplan-Meier analysis and Log-rank test with single-factor analysis for the follow-up data from the 48 BCa patents. We used the median of relative p21 expression as the cut-off value and divided them into high and low p21 expression groups. The median overall survival (OS) after

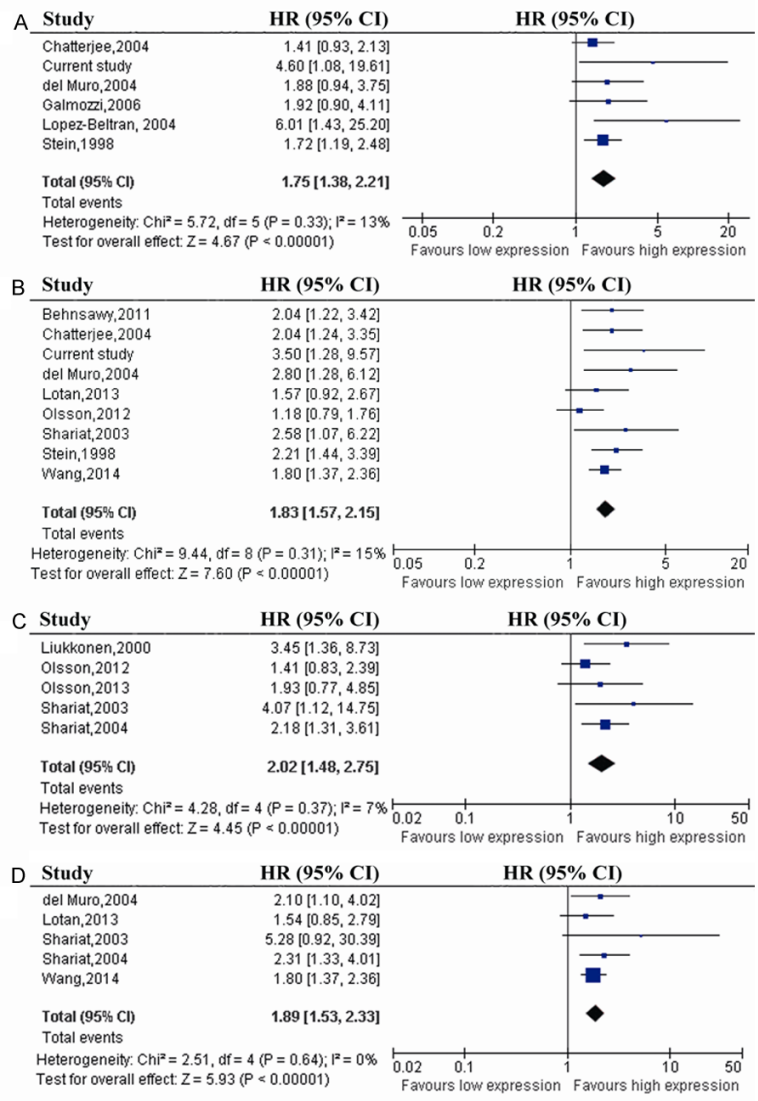

Figure 3. Forest plots of hazard ratios for p21 relative expression in relation to bladder overall survival (A), recurrence-free survival (B), progression free survival (C) and cancer specific survival (D).

cystectomy for the cohort of 48 patients was 39 months (ranging from 1 to 60 months). Kaplan-Meier analysis of patient survival revealed that low expression of p21 may point to highly early recurrence (HR, 3.50; 95% CI, 1.28-9.57; P = 0.016, **Figure 2B**) and poor overall survival (HR, 4.60; 95% CI, 1.08-19.63; P = 0.039, **Figure 2A**) for BCa patients.

Univariate and multivariate analysis of RFS and OS according to clinicopathological factors and p21 expression

Cox regression analysis was used to compare p21 expression with clinicopathological features of survival prediction (**Table 2**). As shown in **Table 2**, using univariate and multivariate Cox regression analysis, the parameters such as tumor grades (G2&3), TNM stage (T2-4), and low p21 expression were significantly associated with poor RFS (P < 0.05) in a cohort of 48 BCa patients. By multivariate Cox regression analysis, only tumor grade and TNM stages were identified to be independent prognostic factors for OS (P < 0.05), while, p21 expression was not significant. Together this showed that p21 together with tumor grade and TNM stages were strongly associated with RFS. However, other variables including age, sex, tumor size and tumor multiplicity seemed to show a general trend, with subtle differences in survival without significance.

Meta-analysis of prognostic value of p21 expression in bladder cancer

Including our current study, a total of 13 studies met the eligibility criteria for this meta-analysis. 9, 6, 5, 5 studies reported p21 expression with RFS, OS, CSS and PFS respectively. Our pooled results showed that p21 expression were statistically significant for overall survival (OS; HR, 1.75; 95% CI, 1.38-2.21; P < 0.001; **Figure 3A**), recurrence-free survival (RFS; HR, 1.83; 95% confidence interval [CI], 1.57-2.15; P < 0.001; **Figure 3B**), progression-free survival (PFS; HR, 2.02; 95% CI, 1.48-2.75; P < 0.001; **Figure 3C**) and cancer-specific survival (CSS; HR, 1.89; 95% CI, 1.53-2.33; P < 0.001; **Figure 3D**). For p21 expression in bladder cancer, no funnel plot asymmetry and no indication of an obvious for publication bias (**Figure 4**).

Discussion

BCa is currently one of the tumors with the most rapid increase in incidence. The traditional management and prognosis of patients with

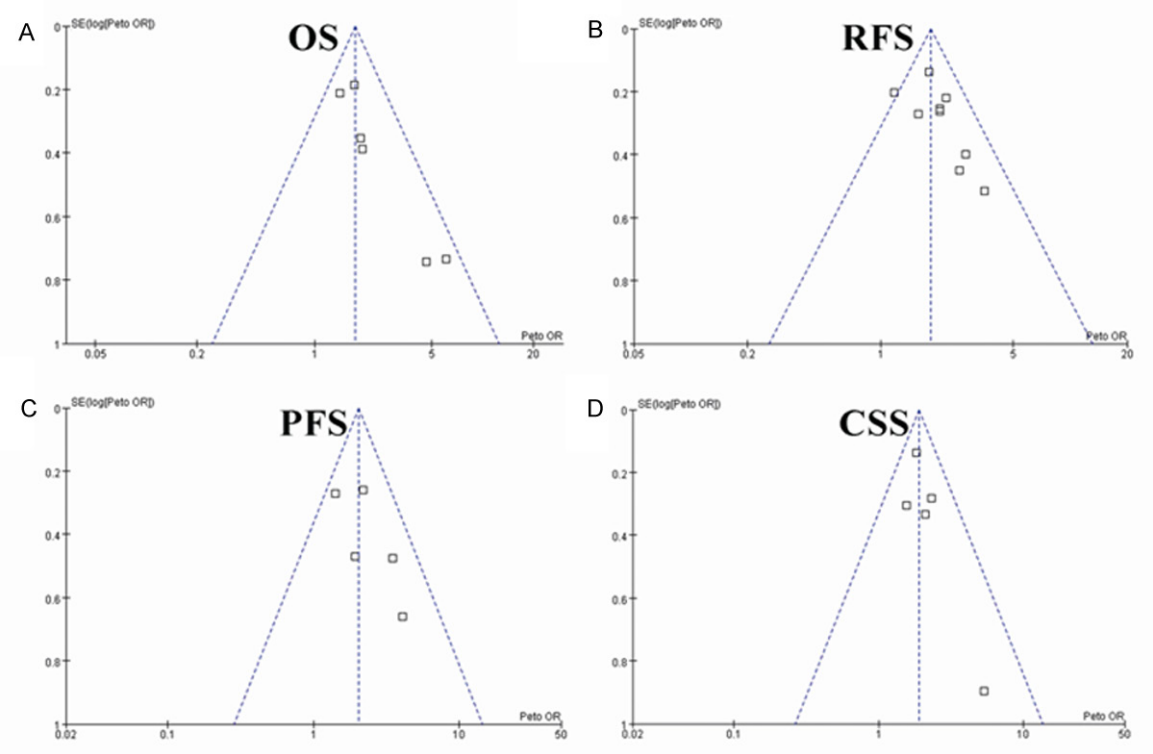

Figure 4. Funnel plots of p21 relative expression in relation to bladder cancer overall survival (A), recurrence-free survival (B), progression free survival (C) and cancer specific survival (D).

bladder cancer is mainly based on clinicopathological parameters. The importance of biomarkers has been recognized and novel biomarker discovery efforts are now being commonly conducted. Yet despite intensified interest and investment, few novel biomarkers are used in clinical practice [22]. Several biomarkers have been proposed to monitor this disease, including individual cell cycle-related protein p21 [23]. Genetic alternations could promote BCa progression, and p21 loss-of-function mutation is one of the most important and plays an important role in carcinogenesis [24]. p21 is a well-known tumor suppressor gene by inhibiting cell proliferation, controlling cell cycle, inducing apoptosis and senescence [25], and attenuating cell invasion and migration [26].

p21 as a tumor suppressor gene has a significant impact in predicting outcomes of BCa, and altered genetic p21 is associated with poor prognosis in BCa which indicated that p21 is involved in the tumorgenesis of cancer progression. We validated p21 expression and conducted the univariate and multivariate analysis of the recurrence free survival and overall survival according to clinicopathological factors and p21 expression in our cohort of 48 BCa patients, which also demonstrated these conclusions. Furthermore, we performed a meta-analysis approach to demonstrate p21 as an independent prognostic biomarker in repeated studies, which complentary to the traditional clinicopathologic factors in patients with BCa. Our present study has provided a better understanding of the association between the altered p21 expression and cancer progression, and also p21 served as a useful biomarker of disease outcome for urological cancers and it is measured objectively and affordably in clinical practice in the future.

The prognostic value of the cyclin-dependent kinase inhibitor p21 has been widely debated. Although it is transcriptionally controlled by p53 [27], this protein has also been shown to be regulated in a p53-independent manner [28]. Absence of expression of p21 plays independent prognostic role for the tumor recurrence and reduced overall survival, as well as demonstrating synergistic effects in tumors with altered p53 expression [19, 29].

Underexpression of p21 has been associated with poor prognostic outcome in BCa. The study by Behnsawy et al [21] included 161 consecutive patients with BCa reported high p21 expression in 78.3% of patients. Univariate and multivariate analysis demonstrated a significant association between lack of p21 immunoreactivity and poor recurrence free survival ($P < 0.05$). Chatterjee et al [9] reported alteration p21 expression, which examined immunohistochemically on archival radical cystectomy samples from 164 patients, in act in cooperative or synergistic ways to promote bladder cancer progression. Conversely, a cohort of 207 patients revealed that immunostaining for p21 did not provide any additional prognostic information compared to established prognostic factors [12]. Furthermore, contradictory results showed that positive p21 expression is associated with recurrence and progression in a multivariate analysis in noninvasive cancer in situ and that the risk increases in synergy with altered p53 expression [17]. Additionally, elevated p21/high Ki-67 expression was demonstrated to be an independent predictor of poor overall survival in invasive BCa [11]. It has been proposed that progressive cancers accumulate p21 due to impairment of the p21-induced inhibitory pathway or mutations in the gene, resulting in the overproduction of abnormal genes [11]. In summary, the use of this cell cycle-related protein as a prognostic biomarker has been extensively studied, but it still remains ambiguous. However, our validated patients with low p21 expression had shorter recurrence-free survival and overall survival rates. Multivariate analysis revealed that p21 low expression was an independent prognostic factor for recurrence free survival. Moreover, in our present meta-analysis, p21 appears to add prognostic information, indicating that they may contribute to more accurate management and predication of BCa.

However, we should admit that there existed several inherent limitations in the trials included in our present study when interpreting our conclusion. The major limitation is that our findings are based on the limitations of the method IHC itself. Although this is a quick, cost-efficient and easy way to measure the expression of a protein of interest and validate it to clinical follow-up, the technique still has limitations. The primary antibody may cross-react with other similar which cause false-positive staining. Post-translational modifications may interfere with the binding, which cause false-negative staining. Second, here we performed a meta-analysis between p21 expression and BCa survival. All of the eligible studies in IHC, while our present study detected miRNAs expression using qRT-PCR. Third, baseline heterogeneity was observed because of methodological and demographic differences among studies examining p21 and prognosis in BCa. The cut-off values of p21 varied greatly between the studies may be the main reason for this heterogeneity. For routine application of biomarkers in clinical use, relevant cutoff values need to be defined. While different cutoff values could be used for the same biomarker depending on the situation. Whatever, here we used appropriate well-motivated inclusion criteria and a comprehensive and robust search strategy to conduct a meta-analysis of HR for survival. We provide the evidence of prognostic significant role of p21 expression in BCa which may worth reference on the clinical follow-up.

Conclusions

In summary, cell cycle-related protein p21 improved the prediction of recurrence and survival after radical cystectomy. Such a biomarker may help identify patients who might benefit from additional treatments and closer surveillance after cystectomy. However, further studies are needed to validate p21 expression in larger patient cohorts, with patients followed prospectively and with more standardized procedures.

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Zhong Chen and Hua Xu, Department of Urology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China. Tel: 86-27-836-63454; Fax: 86-27-836-63454; E-mail: chenzhongtongji@126.com (ZC); xuhuawhu@163.com (HX)

References

[1] Jacobs BL, Lee CT, Montie JE. Bladder cancer in 2010: how far have we come? CA Cancer J Clin 2010; 60: 244-72.

[2] Fangliu G. [Changing constituents of genitourinary cancer in recent 50 years in Beijing]. Chin Med J 2003; 23: 88-90.
[3] Gakis G, Efstathiou J, Lerner SP, Cookson MS, Keegan KA, Guru KA, Shipley WU, Heidenreich A, Schoenberg MP, Sagaloswky AI, Soloway MS, Stenzl A; International Consultation on Urologic Disease-European Association of Urology Consultation on Bladder C. ICUD-EAU International Consultation on Bladder Cancer 2012: Radical cystectomy and bladder preservation for muscle-invasive urothelial carcinoma of the bladder. Eur Urol 2013; 63: 45-57.
[4] Resnick MJ, Bassett JC, Clark PE. Management of superficial and muscle-invasive urothelial cancers of the bladder. Curr Opin Oncol 2013; 25: 281-8.
[5] Chen M, Huang J, Zhu Z, Zhang J, Li K. Systematic review and meta-analysis of tumor biomarkers in predicting prognosis in esophageal cancer. BMC Cancer 2013; 13: 539.
[6] Xu L, Cai J, Yang Q, Ding H, Wu L, Li T, Wang Z. Prognostic significance of several biomarkers in epithelial ovarian cancer: a meta-analysis of published studies. J Cancer Res Clin Oncol 2013; 139: 1257-77.
[7] Chen Z, Place RF, Jia ZJ, Pookot D, Dahiya R, Li LC. Antitumor effect of dsRNA-induced p21(WAF1/CIP1) gene activation in human bladder cancer cells. Mol Cancer Ther 2008; 7: 698-703.
[8] Wang C, Chen Z, Ge Q, Hu J, Li F, Hu J, Xu H, Ye Z, Li LC. Up-regulation of p21(WAF1/CIP1) by miRNAs and its implications in bladder cancer cells. FEBS Lett 2014; 588: 4654-64.
[9] Chatterjee SJ, Datar R, Youssefzadeh D, George B, Goebell PJ, Stein JP, Young L, Shi SR, Gee C, Groshen S, Skinner DG, Cote RJ. Combined effects of p53, p21, and pRb expression in the progression of bladder transitional cell carcinoma. J Clin Oncol 2004; 22: 1007-13.
[10] Garcia del Muro X, Condom E, Vigues F, Castellsague X, Figueras A, Munoz J, Sola J, Soler T, Capella G, Germa JR. p53 and p21 Expression levels predict organ preservation and survival in invasive bladder carcinoma treated with a combined-modality approach. Cancer 2004; 100: 1859-67.
[11] Galmozzi F, Rubagotti A, Romagnoli A, Carmignani G, Perdelli L, Gatteschi B, Boccardo F. Prognostic value of cell cycle regulatory proteins in muscle-infiltrating bladder cancer. J Cancer Res Clin Oncol 2006; 132: 757-64.
[12] Liukkonen T, Lipponen P, Raitanen M, Kaasinen E, Ala-Opas M, Rajala P, Kosma VM. Evaluation of p21WAF1/CIP1 and cyclin D1 expression in the progression of superficial bladder cancer. Finbladder Group. Urol Res 2000; 28: 285-92.
[13] Lopez-Beltran A, Luque RJ, Alvarez-Kindelan J, Quintero A, Merlo F, Requena MJ, Montironi R. Prognostic factors in survival of patients with stage Ta and T1 bladder urothelial tumors: the role of G1-S modulators (p53, p21Waf1, p27Kip1, cyclin D1, and cyclin D3), proliferation index, and clinicopathologic parameters. Am J Clin Pathol 2004; 122: 444-52.
[14] Lotan Y, Bagrodia A, Passoni N, Rachakonda V, Kapur P, Arriaga Y, Bolenz C, Margulis V, Raj GV, Sagalowsky AI, Shariat SF. Prospective evaluation of a molecular marker panel for prediction of recurrence and cancer-specific survival after radical cystectomy. Eur Urol 2013; 64: 465-71.
[15] Olsson H, Hultman P, Monsef N, Rosell J, Jahnson S. Immunohistochemical evaluation of cell cycle regulators: impact on predicting prognosis in stage t1 urinary bladder cancer. ISRN Urol 2012; 2012: 379081.
[16] Olsson H, Hultman P, Rosell J, Soderkvist P, Jahnson S. MDM2 SNP309 promoter polymorphism and p53 mutations in urinary bladder carcinoma stage T1. BMC Urol 2013; 13: 5.
[17] Shariat SF, Kim J, Raptidis G, Ayala GE, Lerner SP. Association of p53 and p21 expression with clinical outcome in patients with carcinoma in situ of the urinary bladder. Urology 2003; 61: 1140-5.
[18] Shariat SF, Tokunaga H, Zhou J, Kim J, Ayala GE, Benedict WF, Lerner SP. p53, p21, pRB, and p16 expression predict clinical outcome in cystectomy with bladder cancer. J Clin Oncol 2004; 22: 1014-24.
[19] Stein JP, Ginsberg DA, Grossfeld GD, Chatterjee SJ, Esrig D, Dickinson MG, Groshen S, Taylor CR, Jones PA, Skinner DG, Cote RJ. Effect of p21WAF1/CIP1 expression on tumor progression in bladder cancer. J Natl Cancer Inst 1998; 90: 1072-9.
[20] Wang LC, Xylinas E, Kent MT, Kluth LA, Rink M, Jamzadeh A, Rieken M, Al Awamlh BA, Trinh QD, Sun M, Karakiewicz PI, Novara G, Chrystal J, Zerbib M, Scherr DS, Lotan Y, Vickers A, Shariat SF. Combining smoking information and molecular markers improves prognostication in patients with urothelial carcinoma of the bladder. Urol Oncol 2014; 32: 433-40.
[21] Behnsawy HM, Miyake H, Abdalla MA, Sayed MA, Ahmed Ael F, Fujisawa M. Expression of cell cycle-associated proteins in non-muscle-invasive bladder cancer: correlation with intravesical recurrence following transurethral resection. Urol Oncol 2011; 29: 495-501.
[22] Bensalah K, Montorsi F, Shariat SF. Challenges of cancer biomarker profiling. Eur Urol 2007; 52: 1601-9.
[23] Rosenblatt R, Jonmarker S, Lewensohn R, Egevad L, Sherif A, Kalkner KM, Nilsson S,

Valdman A, Ullen A. Current status of prognostic immunohistochemical markers for urothelial bladder cancer. Tumour Biol 2008; 29: 311-22.

[24] Cancer Genome Atlas Research N. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature 2014; 507: 315-22.

[25] Warfel NA, El-Deiry WS. p21WAF1 and tumourigenesis: 20 years after. Curr Opin Oncol 2013; 25: 52-8.

[26] Dai M, Al-Odaini AA, Fils-Aime N, Villatoro MA, Guo J, Arakelian A, Rabbani SA, Ali S, Lebrun J. Cyclin D1 cooperates with p21 to regulate TGF-beta-mediated breast cancer cell migration and tumor local invasion. Breast Cancer Res 2013; 15: R49.

[27] el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B. WAF1, a potential mediator of p53 tumor suppression. Cell 1993; 75: 817-25.

[28] Michieli P, Chedid M, Lin D, Pierce JH, Mercer WE, Givol D. Induction of WAF1/CIP1 by a p53-independent pathway. Cancer Res 1994; 54: 3391-5.

[29] Sgambato A, Migaldi M, Leocata P, Ventura L, Criscuolo M, Di Giacomo C, Capelli G, Cittadini A, De Gaetani C. Loss of p27Kip1 expression is a strong independent prognostic factor of reduced survival in N0 gastric carcinomas. Cancer 2000; 89: 2247-57.