Int J Clin Exp Med 2016;9(12):23482-23490
www.ijcem.com /ISSN:1940-5901/IJCEM0034516

Original Article

Cyclin D1 expression is associated with stage, grade and survival in urinary bladder carcinoma

Mohamad Nidal Khabaz[1], Abdelbaset Buhmeida[2], Tawfik Ghabrah[3], Imtiaz Ahmad Qureshi[1], Nadeem Shafique Butt[3], Basim Al-Maghrabi[4], Taoufik Nedjadi[5], Mohmmad Al-Qahtani[2], Jaudah Al-Maghrabi[6]

[1]Department of Pathology, Rabigh Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia; [2]Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Saudi Arabia; [3]Department of Family and Community Medicine, Rabigh Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia; [4]Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia; [5]King Fahd Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia; [6]Department of Pathology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

Received June 27, 2016; Accepted September 5, 2016; Epub December 15, 2016; Published December 30, 2016

Abstract: This study investigates the association between cyclin D1 immunohistochemical phenotype and the clinicopathological findings in bladder cancer. One hundred and twenty eight cases of previously diagnosed bladder cancer and 24 tissue samples of normal bladder were utilized for cyclin D1 expression detection using tissue microarrays and immunohistochemistry. High grading score of nuclear cyclin D1 immuno expression has been found in 66 (51.6%) bladder cancer cases, while 12 (50%) control cases showed cyclin D1 immunoreactivity. Strong cyclin D1 immunohistochemical staining has been significantly linked with low grades (P=0.001), low stages (P=0.005), while low scores of cyclin D1 immunostaining were associated with muscularis propria invasion (P=0.003), lymph node invasion (P=0.024), and vascular invasion (P=0.010). Furthermore, histotype of bladder cancer slightly associated with cyclin D1 immunostaining (P=0.049), all of the squamous cell carcinoma cases showed low level of cyclin D1 immunostaining, while 55.4% of urothelial carcinoma cases revealed strong cyclin D1 immunostaining. Significant different survival distributions have been observed with cyclin D1 staining of transformed epithelium (P=0.026). High cyclin D1 staining of transformed epithelial cells is positively associated with poor survival. Our results confirm the great values of cyclin D1 in the prognosis of bladder cancer. These preliminary findings recommend that cyclin D1 maybe a valuable tissue biomarker for presaging grade, stage, and poor prognosis in bladder cancer.

Keywords: Cyclin D1, bladder cancer, immunohistochemistry

Introduction

Bladder cancer (BC) is a devastating disease and an important cause of cancer death around the world [1]. According to a recently published data, BC is ranked sixth in the list of most frequent tumors with around 74,000 new registered cases and more than 15,000 bladder cancer deaths in USA in 2015 [2]. Despite important advances in cancer treatment, the disease continues to pose a big challenge to clinicians due to high recurrence rates, as 50-70% of new cancer cases recur within 5 years, and a great likelihood to progress to an aggressive, muscle invasive and metastatic forms [3, 4]. The management of BC relies enormously on the patients' clinicopathological parameters, such as TNM staging and grade of the tumor, as indicators of good/poor prognosis. However, these parameters are not sufficient to predict patients' outcome and worse yet, produce large discrepancies within the same grade/stage. This is mainly related to heterogeneity of bladder cancer cells [5]. Hence there is an urgent need to develop new diagnostic and therapeutic modalities to meet clinical needs for bladder cancer management; intensive works are being made to develop novel biochemical markers so as to assist in the diagnosis and prognosis of the tumor, enhance stratification of high risk patients and improve clinical management [6, 7]. Most of these tools have not been sufficiently sensitive or specific which necessitate the identification

of additional robust biomarkers that would more accurately predict for patient's prognosis and improve therefore BC surveillance in clinical setting.

Cyclin D1 is a member of G1 family of cyclins. Its coding gene located on band q13 of chromosome 11 [8, 9]. Cyclin D1 conducts a significant task in the cell cycle progression; it influences positively the progress G1-S phase in cell cycle [10]. Therefore this protein is thought a possible oncogene. Cyclin D1 gene amplification or its genomic alteration, which may cause protein overexpression, are frequently realized as a clonal pathology in various human neoplasms. These changes in cyclin D1 gene suggest the significance of overexpressed cyclin D1 as a player in the transformation process [11]. Cyclin D1 shows a leading effect in controlling proliferation, conveying extracellular signals into cell cycle progression [12]. The quantity of cyclin D1 expression is greatly reactive to the influence of proliferation activators such as growth factors and Ras [13]. Overexpression of cyclin D1 could be the consequence of CCND1 amplification or rearrangement [14]. Amplifications of CCND1 have been described in numerous neoplasms, for instance prostate, bladder, breast, lung, and head and neck cancer [15]. Moreover, cyclin D1 overexpression may occur without the amplification of its coding gene in large number of colorectal carcinomas and to a lesser extent in breast carcinomas [16]. Cyclin D1 defective degradation has been suggested as a protein overexpression mechanism in some tumors [17]. This protein has been considered as an oncoprotein in various tumors once overexpressed. Many investigators reported cyclin D1 oncogenic effects and its overexpression. Furthermore, they linked cyclin D1 with diagnosis, prognosis and survival in different tumors. Cyclin D1 overexpression has been documented in breast cancer [18], ovarian cancer [19], esophageal cancer [20], non-small cell lung carcinomas [21], and endometrial carcinoma [22]. Therefore, many studies attempted to prove that cyclin D1 expression phenotype could be utilizedas a diagnostic and prognostic tissue marker in bladder tumors; however their findings need further confirmation [23-44].

In the current research study, we evaluated cyclin D1 immunohistochemical phenotype in bladder cancer in comparison with normal bladder tissue, and assessed the association of cyclin D1 immunoreactivity with clinicopathological findings and survival in the west province of Saudi Arabia.

Material and methods

Subjects

One hundred and twenty eight tumors of bladder have been recovered from the archives of the Department of Pathology in King Abdulaziz University Hospital, Kingdom of Saudi Arabia. Furthermore, 24 cases of normal bladder were utilized as controls. Paraffin tissue blocks were sectioned and H & E stained for tumor histological evaluations. The clinical data of patients such as age, histotype, size, stage and grade were retrieved from the unit of medical records (**Table 1**). World Health Organization recommendation was used for grading and staging of bladder cancer. All tissue blocks of tumors and controls were employed for tissue microarray production. The present study has been conducted according to the obtained approval from Ethical Committee at King Abdulaziz University.

Tissue microarray production

One hundred and twenty eight cases of bladder cancer and 24 samples of normal bladder were utilized for the production of tissue microarray (TMA) as we reported in our previous study [45]. TMA blocks have been sectioned and put on coated slides, then they have been subjected to cyclin D1 immunohistochemical staining.

Immunohistochemical staining method

Multimer technology has been applied in the immunohistochemical staining of the tumor sections of bladder employing VENTANA anti-cyclin D1 (SP4-R) antibody (Ventana, Arizona, USA) and ULTRAVIEW TM DAB visualizing system according to the instruction of kit manufacturer (Ventana, Arizona, USA). BenchMark ULTRA autostainer has been used for automated immunohistochemical staining (Ventana, Arizona, USA). A negative control slide has been added to each staining run. This slide contained trisbuffer instead of anti-cyclin D1 antibody. Slide section of human tonsil tissue has been included as positive control. Cases with nuclear brown immunostaining in more than 5% of neoplastic cells were counted positive.

Table 1. Distribution of various clinicopathological variables of bladder cancer with cyclin D1 immunostaining levels

		Cyclin D1 immunostaining score				*P*-Value
		Low		High		
		Count	Row N%	Count	Row N%	
Gender (Male/Female)	Female	11	45.8%	13	54.2%	0.824
	Male	51	49.0%	53	51.0%	
Histotype of Cancer (Transitional or Squamous)	Squamous	6	100.0%	0	0.0%	0.049
	Transitional	45	44.6%	56	55.4%	
	Transitional/CIS	2	50.0%	2	50.0%	
	Transitional/Squamous	9	52.9%	8	47.1%	
Grade	High Grade	38	58.5%	27	41.5%	0.001
	Low Grade	15	29.4%	36	70.6%	
	NA	9	75.0%	3	25.0%	
Stage	0a	4	23.5%	13	76.5%	0.005
	0is	3	60.0%	2	40.0%	
	I	9	29.0%	22	71.0%	
	II	22	57.9%	16	42.1%	
	III	5	71.4%	2	28.6%	
	IV	15	75.0%	5	25.0%	
	Undecided	4	40.0%	6	60.0%	
Muscularis propria Invasion	NON	15	31.9%	32	68.1%	
	Positive	40	63.5%	23	36.5%	0.003
	Undecided	7	38.9%	11	61.1%	
Vascular Invasion	NON	48	43.6%	62	56.4%	0.010
	Positive	14	77.8%	4	22.2%	
Lymph Node	NON	48	44.0%	61	56.0%	0.024
	Positive	14	73.7%	5	26.3%	
Distant Metastases	NON	47	44.3%	59	55.7%	0.060
	Positive	15	68.2%	7	31.8%	
Alive/Deceased status	ALIVE	40	44.4%	50	55.6%	0.181
	DEAD	22	57.9%	16	42.1%	

Cyclin D1 immunoreactivity has been scored by two pathologists for staining intensity and positively stained cells percentage. The frequency of positively stained cells has been evaluated using semi-quantitative approach in three 400 magnification fields. Cyclin D1 positively stained cases have been scored for staining intensity considering strong =3, moderate =2, weak staining =1, and negative =0 [45]. Staining intensity scores has been introduced as low level immunoreactivity (0 and 1) and high level (2 and 3) in the current study. When a difference between the scores of the two pathologists has occurred, the smallest grade of staining was recorded.

Statistical analysis

IBM-SPSS version 21 has been employed for results analyses. Chi-Square and Fisher's exact test have been applied to explore the relationship of cyclin D1 immunostaining with various clinicopathological variables of bladder cancer. Log Rank (Mantel-Cox) test and Kaplan Meier survival curves have been applied to compare survival distributions for the different levels of Cyclin D1 immunostaining. $P<0.05$ has been presented as statistically significant.

Results

One hundred and twenty eight cases of bladder cancer (104 males and 24 females) were revised. Both genders showed almost similar distribution pattern of immunohistochemical staining. Clinicopathological parameters of these cases have been presented in **Table 1**. The most common type was urothelial carcinoma (78.9%) and less frequently squamous differentiation variant (13.3%), squamous cell carcino-

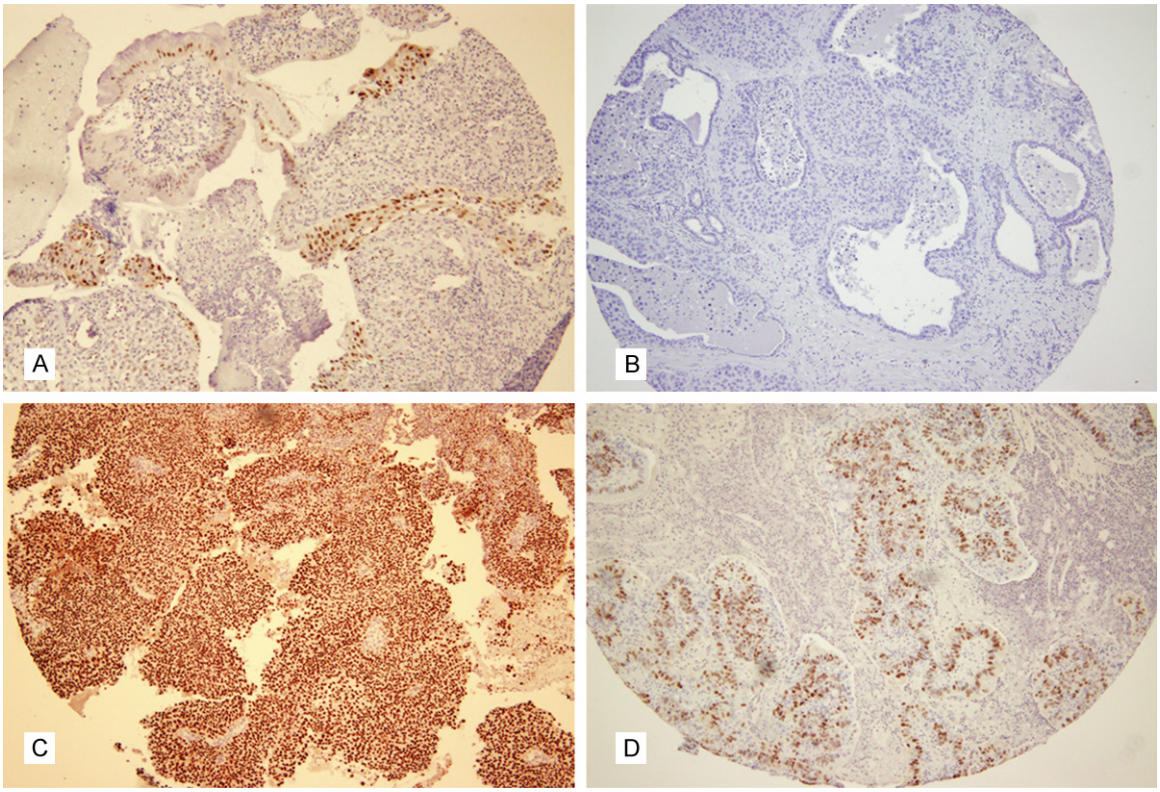

Figure 1. Nuclear cyclin D1 expression pattern in bladder cancer. A. Strong cyclin D1 immunohistochemical staining in normal bladder tissue (10 X); B. Negative cyclin D1 immunohistochemical stained bladder cancer (10 X); C. Strong cyclin D1 immunohistochemical stained bladder cancer (10 X); D. Moderate positive cyclin D1 staining in bladder cancer (10 X).

ma (4.7%), and carcinoma in situ (3.1%) (**Table 1**). The average age in the present study was 62.4 years (ranged 31-93 yrs.). One hundred and eighteen cases of bladder cancer have been found to be staged and 116 cases ungraded (**Table 1**). The total number of deaths from bladder cancer was 38 (29.7%). Sixty three cases showed muscularis propria invasion, eighteen cases with vascular invasion, nineteen cases with lymph node involvement and twenty two cases with distant metastases (**Table 1**).

Cyclin D1 immunohistochemical staining was found significantly correlated with the histotype of bladder tumor, grades, stages, muscularis propria invasion, vascular invasion and lymph node invasion. Histotypes of bladder cancer were significantly linked with cyclin D1 staining intensity (P=0.049). Strong nuclear immunostaining of cyclin D1 was foundin 66 (51.6%) cases of bladder cancer which include 56 (55.4%) cases of urothelial carcinoma (**Figure 1**), 2 (50%) urothelial carcinomas in situas well as 8 (47.1%) cases of squamous differentiation variant. While all cases of squamous cell carcinoma showed low scores of cyclin D1 immunoreactivity. More than 65% of the cases showed immunostaining in more than fifty percent of the transformed cells. The majority of low grade tumors displayed strong cyclin D1 immunostaining (P=0.001) whereas low level of cyclin D1 immunoreactivity was more common in tumors with high grade (**Table 1**). Tumor stage showed also significant association with cyclin D1 immunostaining (P=0.005), a considerable fraction of low stage tumors had higher cyclin D1 immunostaining scores whereas lowcyclin D1 immunoreactivity is more frequent in high stage (III and IV) tumors (**Table 1**). Muscularis propria invasion, vascular invasion and lymph node invasion occurred more frequently in bladder cancersin which their cells exhibited low score for cyclin D1 immunostaining (P= 0.003, P=0.010, P=0.024 respectively). Furthermore, most bladder tumors which did not invade muscularis propria, blood vessels and lymph nodes showed high level of cyclin D1

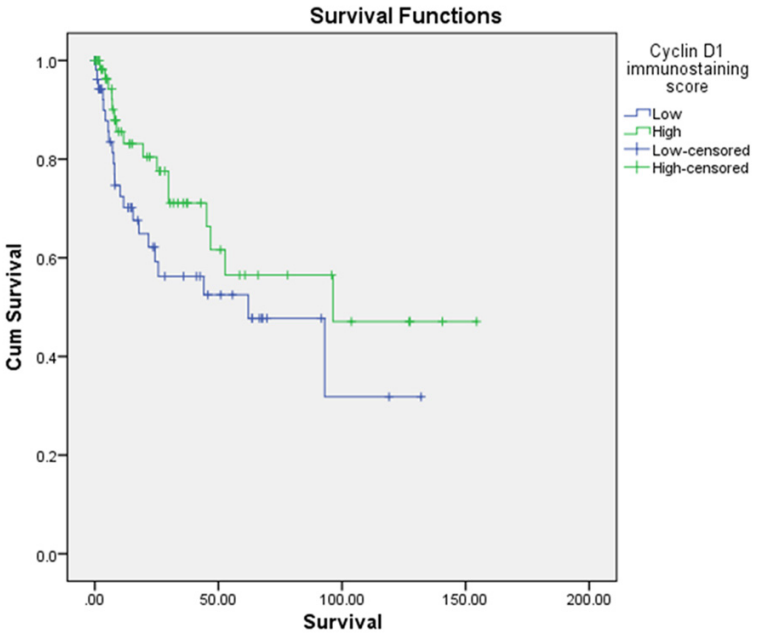

Figure 2. Kaplan Meier survival curves by pattern of cyclin D1 immunostaining shows significantly poor survival behavior associated with cyclin D1 immunostaining in bladder cancer.

immunoreactivity. No significant associations were observe with gender, presence of distant metastases and alive/deceased status.

The result of log-rank (Mantel-Cox) test for likeness of survival distributions for various pattern of cyclin D1 immunostaining did not show significant different survival distribution (P=0.117). **Figure 2** shows significantly poor survival behavior with low level of cyclin D1 immunoreactivity so it can be said that low score cyclin D1 immunostaining is positively related to poor survival.

Twelve (50%) control cases showed nuclear cyclin D1 immunostaining. There was no statistical variation in cyclin D1 expression observed among tumor cases and control group.

Discussion

Cyclin D1 regulates the G0-G1 succession as well as promotes cell growth. Inconsistent information on the significance of this protein have been stated in the subject of bladder cancer (**Table 2**) [23-44]. In the current study, immunoreactivity of cyclin D1 was found similarly frequent in bladder tumors (51.6%) and normal tissue of bladder (50%) with no statistical significant difference; however, the present results are consistent with those of other studies [23, 39, 43] which documented almost similar frequency of positive cyclin D1 immunostaining in bladder tumors (**Table 2**). The level of Cyclin D1 immunoreactivity was associated with good and poor prognosis [36, 37]. Nevertheless, most of the reports regarding immunohistochemical phenotype of cyclin D1, performed in bladder malignancy, have involved cases of low stage bladder cancer with no muscularis propria invasion (NMIBC). The current report has investigated bladder cancer with muscularis propria invasion (MIBC) and NMIBC. It was found that high level of cyclin D1 immunoreactivity is more frequent in NMIBC while high stage and grade tumors, MIBC tumors and tumors with vascular invasion and lymph node involvement showed more frequently low level of immunohistochemical scores.

Although, at present, there is neither single tissue biomarker that is able to predict the clinical outcomes of bladder tumors nor markers developed from the bladder cancer pathogenesis can be respected to recognize individuals at risk for tumor progression. Our results have established significant associations of cyclin D1 expression with histotype, grade, stage, muscularis propria invasion, lymph node invasion, vascular invasion, and poor survival of bladder tumors. Furthermore, these outcomes are consistent with the findings of many other studies [28, 31, 34, 38-43] which have stated that cyclin D1 expression was correlated with low grade and stage tumors (**Table 2**). However, they contradicted the conclusions of Xu et al. [23], Seiler et al. [24], Lee et al. [30], Shariat et al. [33] and Sgambato et al. [37] who did not find similar association in bladder malignancy.

It has been accepted that high grade neoplasms of bladder have higher progression and invasiveness rate more than low-grade tumors [46]. In this study, the phenotype of cyclin D1

Table 2. Correlation between high level of cyclin D1 immunoreactivity and clinicopathological parameters in the current study compared to previous studies of the literature

Previous studies	Cyclin D1 in Bladder Cancer	Cyclin D1 in Normal Bladder	Histotype	Grade	Stage	Recurrence	Muscularis propria NMIBC vs. MIBC	Vascular Invasion	Lymph Node	Distant Metastases	Alive/Deceased status	Survival
The current study	51.6%		P=0.049	Low grades P=0.001	Low stages P=0.005		NMIBC P=0.003	Inverse relation P=0.010	Inverse relation P=0.024	Not significant	Not significant	Weak staining Poor survival
Xu et al. [23]	56.5% P=001	12.5%		High grades 69.8% P=0.001	High stages 76.9% P=0.012	70.3% P=0.024	MIBC 76.9% P=0.012					Strong staining Poor survival
Seiler et al. [24]					Not significant				Not significant	Not significant		Strong staining Poor survival
Fristrup et al. [25]		Negative					MIBC P<0.001					
Kopparapu et al. [26]	88.3% P=0.001	81.7%				Not significant	NMIBC P=0.001		P<0.001			
Olsson et al. [27]	71.%					Not significant	Not significant		Not significant	Not significant	Not significant	
Lenz et al. [28]				Low grades P=0.002	Low stages P=0.0007							
Behnsawy et al. [29]						Not significant						
Lee et al. [30]	19.5%				High stages P=0.017	Inverse relation P=0.036					Not significant	
Levidou et al. [31]	99.36%		Not significant	Low grades P=0.0001	Low stages P=0.0001		MIBC P=0.0033					Weak staining Poor survival
Brunner et al. [32]	39%					Not significant	MIBC P=0.032		P=0.032	P=0.032		
Shariat et al. [33]	69%	100%		Not significant	Not significant	Not significant	Not significant		Not significant	Not significant	Not significant	
Galmozzi et al. [34]					Low stages P=0.001						Not significant	
Yurakh et al. [35]							P=0.0053		P=0.0053	P=0.0053		
Lopez-Beltran et al. [36]							P<0.001		P<0.001	P<0.001		Strong staining Poor survival
Sgambato et al. [37]				Not significant	Not significant							Strong staining Good survival
Ioachim et al. [38]	26%	Negative		Low grades P=0.05	Low stages P=0.021							
Mhawech et al. [39]	48%			Low grades P<0.001	Low stages P=0.002							
Tut et al. [40]	83%			Low grade P<0.005	Low stage P<0.005							Weak staining Poor survival
Takagi et al. [41]	77%	Negative		Low grades P<0.0001	Low stages P<0.0001	Not significant						Weak staining Poor survival
Liukkonen et al. [42]	75%			Low grades P=0.006	Low stages P=0.001	P=0.04	MIBC P=0.014					
Wagner et al. [43]	45%		P<0·005	Low grades P<0·005	Low stages P<0·005	Not significant	Not significant		Not significant	Not significant		
Shin et al. [44]	25.3%			Not significant		P<0.01						Not related

was correlated with the degree of cancer progression and invasiveness. Absenceor low score of cyclin D1 immunoexpression in bladder neoplasm was found to be related to muscularis propria invasion, vascular invasion, lymph node invasion. Since nucleus is the ordinary location of this protein in normal cells, changed expression or localization of cyclin D1 may lead to changes in the biological behavior of transformed cells, for instance growth, proliferation, invasion and survival [47]. Our findings recommend the usage of cyclin D1 as a possible biomarker for the stratification of bladder tumor subtypes for personalized therapy.

The differences between the previous studies and the current one could be clarified by methods sensitivity, people's difference, and variations in the size of samples. The present report and previous similar ones which evaluated the diagnostic and prognostic power of cyclin D1 immunoreactivity in bladder malignancy had weakness points such as the relatively small sample size involved in these studies and the semi-quantitative interpretation of immunostaining. However, greater inclusive studies are undoubtedly of great value for estimating the diagnostic and prognostic values of cyclin D1 immunoreactivity in bladder malignancy.

The findings of the current study support using cyclin D1 to support the diagnosis and prognosis of bladder tumor. These preliminary findings recommend cyclin D1 as a valuable tissue biomarker for predicting histotype, grade, stage, progression and prognosis in bladder neoplasms. The correlation of cyclin D1 with several clinicopathological parameters suggests the involvement of this molecule in bladder cancer progression.

Acknowledgements

This project was funded by the National Plan for Science, Technology and Innovation (MAARIFAH)-King Abdulaziz City for Science and Technology-the Kingdom of Saudi Arabia-award number (11-BIO1524-03). The authors also, acknowledge with thanks Science and Technology Unit, King Abdulaziz University for technical support.

Disclosure of conflict of interest

None.

Address correspondence to: Jaudah Al-Maghrabi, Department of Pathology, King Abdulaziz University, P.O. Box 80205, Jeddah 21589, Saudi Arabia. Tel: +9662 6401000. Ext. 17069; Fax: +966 2 64010-00. Ext. 17223. E-mail: jalmaghrabi@hotmail.com; jalmgrabi@kau.edu.sa

References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.

[3] Sylvester RJ, van der Meijden AP, Oosterlinck W, Witjes JA, Bouffioux C, Denis L, Newling DW, Kurth K. Predicting Recurrence and Progression in Individual Patients with Stage Ta T1 BladderCancer Using EORTC Risk Tables: A Combined Analysis of 2596 Patients from Seven EORTC Trials. Eur Urol 2006; 49: 466-477.

[4] Prout GR Jr, Barton BA, Griffin PP, Friedell GH. Treated history of noninvasive grade 1 transitional cell carcinoma. The National Bladder Cancer Group. J Urol 1992; 148: 1413-1419.

[5] Youssef RF, Lotan Y. Predictors of Outcome of Non-Muscle-Invasive and Muscle-Invasive Bladder Cancer. Sci World J 2011; 11: 369-381.

[6] Cheung G, Sahai A, Billia M, Dasgupta P, Khan MS. Recent advances in the diagnosis and treatment of bladder cancer. BMC Med 2013; 11: 13.

[7] Kelloff GJ, Sigman CC, Scher HI. Biomarker development in the context of urologic cancers. Urol Oncol 2015; 33: 295-301.

[8] Bates S, Peters G. Cyclin D1 as a cellular protooncogene. Semin Cancer Biol 1995; 6: 73-82.

[9] Motokura T, Bloom T, Kim HG, Jüppner H, Ruderman JV, Kronenberg HM, Arnold A. A novel cyclin encoded by a bcl1-linked candidate oncogene. Nature 1991; 350: 512-515.

[10] Lukas J, Muller H, Bartkova J, Spitkovsky D, Kjerulff AA, Jansen-Durr P, Strauss M, Bartek JJ. DNA tumor virus oncoproteins and retinoblastoma gene mutations share the ability to relieve the cell's requirement for cyclin-D1 function in G1. Cell Biol 1994; 125: 625-638.

[11] Bartek J, Lukas J, Bartkova J. Perspective: defects in cell cycle control and cancer. J Pathol 1999; 187: 95-99.

[12] Sherr CJ. D-Type Cyclins. Trends Biochem Sci 1995; 20: 187-190.

[13] Aktas H, Cai H, Cooper GM. Ras links growth factor signaling to the cell cycle machinery via regulation of cyclin D1 and the cdk inhibitor p27kip1. Mol Cell Biol 1997; 17: 3850-3857.

[14] Campo E, Raffeld M, Jaffe ES. Mantle-cell lymphoma. Semin Hematol 1999; 36: 115-27.

[15] Schraml P, Kononen J, Bubendorf L, Moch H, Bissig H, Nocito A, Mihatsch MJ, Kallioniemi OP, Sauter G. Tissue microarrays for gene amplification surveys in many different tumor types. Clin Cancer Res 1999; 5: 1966-75.
[16] Russell A, Thompson MA, Hendley J, Trute L, Armes J, Germain D. Cyclin D1 and D3 associate with the SCF complex and are coordinately elevated in breast cancer. Oncogene 1999; 18: 1983-91.
[17] Alt JR, Cleveland JL, Hannink M, Diehl JA. Phosphorylation-dependent regulation of cyclin D1 nuclear export and cyclin D1-dependent cellular transformation. Genes Dev 2000; 14: 3102-14.
[18] Li Z, Cui J, Yu Q, Wu X, Pan A, Li L. Evaluation of CCND1 amplification and CyclinD1 expression: diffuse and strong staining of CyclinD1 could have same predictive roles as CCND1 amplification in ER positive breast cancers. Am J Transl Res 2016; 8: 142-53.
[19] Kanter M, Turan G, Usta C, Usta A, Esen HH, Tavlı L, Celik C, Demirkol Y, Kanter B. Survivin and cycline D1 expressions are associated with malignant potential in mucinous ovarian neoplasms. J Mol Histol 2016; 47: 145-52.
[20] Hou X, Liang RB, Wei JC, Xu Y, Fu JH, Luo RZ, He JH, Zhang LJ, Lin P, Yang HX. Cyclin D1 expression predicts postoperative distant metastasis and survival in resectable esophageal squamous cell carcinoma. Oncotarget 2016; 7: 31088-96.
[21] Lee E, Jin D, Lee BB, Kim Y, Han J, Shim YM, Kim DH. Negative effect of cyclin D1 overexpression on recurrence-free survival in stage II-IIIA lung adenocarcinoma and its expression modulation by vorinostat in vitro. BMC Cancer 2015; 15: 982.
[22] Kala M, Gill M, Gupta V, Srivastava D, Tanwar P and Goyal V. Cyclin D1 Expression in Hyperplasia and Carcinoma of the Endometrium and Its Correlation with Histologic Grade and Tumor Type. J Gynecol Surg 2014; 30: 1-4.
[23] Xu S, Gu G, Ni Q, Li N, Yu K, Li X, Liu C. The expression of AEG-1 and Cyclin D1 in human bladder urothelial carcinoma and their clinicopathological significance. Int J Clin Exp Med 2015; 8: 21222-8.
[24] Seiler R, Thalmann GN, Rotzer D, Perren A, Fleischmann A. CCND1/CyclinD1 status in metastasizing bladder cancer: a prognosticator and predictor of chemotherapeutic response. Mod Pathol 2014; 27: 87-95.
[25] Fristrup N, Birkenkamp-Demtröder K, Reinert T, Sanchez-Carbayo M, Segersten U, Malmström PU, Palou J, Alvarez-Múgica M, Pan CC, Ulhøi BP, Borre M, Ørntoft TF, Dyrskjøt L. Multicenter validation of cyclin D1, MCM7, TRIM29, and UBE2C as prognostic protein markers in non-muscle-invasive bladder cancer. Am J Pathol 2013; 182: 339-49.
[26] Kopparapu PK, Boorjian SA, Robinson BD, Downes M, Gudas LJ, Mongan NP, Persson JL. Expression of cyclin d1 and its association with disease characteristics in bladder cancer. Anticancer Res 2013; 33: 5235-42.
[27] 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.
[28] Lenz P, Pfeiffer R, Baris D, Schned AR, Takikita M, Poscablo MC, Schwenn M, Johnson A, Jones M, Kida M, Cantor KP, Rothman N, Silverman DT, Hewitt SM, Moore LE. Cell-cycle control in urothelial carcinoma: large-scale tissue array analysis of tumor tissue from Maine and Vermont. Cancer Epidemiol Biomarkers Prev 2012; 21: 1555-64.
[29] 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.
[30] Lee K, Jung ES, Choi YJ, Lee KY, Lee A. Expression of pRb, p53, p16 and cyclin D1 and their clinical implications in urothelial carcinoma. J Korean Med Sci 2010; 25: 1449-55.
[31] Levidou G, Saetta AA, Karlou M, Thymara I, Pratsinis H, Pavlopoulos P, Isaiadis D, Diamantopoulou K, Patsouris E, Korkolopoulou P. D-type cyclins in superficial and muscle-invasive bladder urothelial carcinoma: correlation with clinicopathological data and prognostic significance. J Cancer Res Clin Oncol 2010; 136: 1563-71.
[32] Brunner A, Verdorfer I, Prelog M, Mayerl C, Mikuz G, Tzankov A. Large-scale analysis of cell cycle regulators in urothelial bladder cancer identifies p16 and p27 as potentially useful prognostic markers. Pathobiology 2008; 75: 25-33.
[33] Shariat SF, Ashfaq R, Sagalowsky AI, Lotan Y. Association of cyclin D1 and E1 expression with disease progression and biomarkers in patients with nonmuscle-invasive urothelial cell carcinoma of the bladder. Urol Oncol 2007; 25: 468-75.
[34] 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.
[35] Lopez-Beltran A, Luque RJ, Alvarez-Kindelan J, Quintero A, Merlo F, Carrasco JC, Requena MJ, Montironi R. Prognostic factors in stage T1 grade 3 bladder cancer survival: the role of

G1-S modulators (p53, p21Waf1, p27kip1, Cyclin D1, and Cyclin D3) and proliferation index (ki67-MIB1). Eur Urol 2004; 45: 606-12.

[36] 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.

[37] Ioachim E, Michael M, Stavropoulos NE, Kitsiou E, Hastazeris K, Salmas M, Stefanaki S, Agnantis NJ. Expression patterns of cyclins D1, E and cyclin-dependent kinase inhibitors p21(Waf1/Cip1) and p27(Kip1) in urothelial carcinoma: correlation with other cell-cycle-related proteins (Rb, p53, Ki-67 and PCNA) and clinicopathological features. Urol Int 2004; 73: 65-73.

[38] Mhawech P, Greloz V, Oppikofer C, Szalay-Quinodoz I, Herrmann F. Expression of cell cycle proteins in T1a and T1b urothelial bladder carcinoma and their value in predicting tumor progression. Cancer 2004; 100: 2367-75.

[39] Sgambato A, Migaldi M, Faraglia B, De Aloysio G, Ferrari P, Ardito R, De Gaetani C, Capelli G, Cittadini A, Trentini GP. Cyclin D1 expression in papillary superficial bladder cancer: its association with other cell cycle-associated proteins, cell proliferation and clinical outcome. Int J Cancer 2002; 97: 671-8.

[40] Tut VM, Braithwaite KL, Angus B, Neal DE, Lunec J, Mellon JK. Cyclin D1 expression in transitional cell carcinoma of the bladder: correlation with p53, waf1, pRb and Ki67. Br J Cancer 2001; 84: 270-5.

[41] Takagi Y, Takashi M, Koshikawa T, Sakata T, Ohshima S. Immunohistochemical demonstration of cyclin D1 in bladder cancers as an inverse indicator of invasiveness but not an independent prognostic factor. Int J Urol 2000; 7: 366-72.

[42] 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.

[43] Wagner U, Süess K, Luginbühl T, Schmid U, Ackermann D, Zellweger T, Maurer R, Alund G, Knönagel H, Rist M, Jordan P, Moch H, Mihatsch MJ, Gasser TC, Sauter G. Cyclin D1 overexpression lacks prognostic significance in superficial urinary bladder cancer. J Pathol 1999; 188: 44-50.

[44] Shin KY, Kong G, Kim WS, Lee TY, Woo YN, Lee JD. Overexpression of cyclin D1correlates with early recurrence in superficial bladder cancers. Br J Cancer 1997; 75: 1788-92.

[45] Al-Maghrabi J, Emam E, Gomaa W, Saggaf M, Buhmeida A, Al-Qahtani M, Al-Ahwal M. c-MET immunostaining in colorectal carcinoma is associated with local disease recurrence. BMC Cancer 2015; 15: 676.

[46] Raman JD, Ng CK, Boorjian SA, Vaughan ED Jr, Sosa RE, Scherr DS. Bladder cancer after managing upper urinary tract transitional cell carcinoma: Predictive factors and pathology. BJU Int 2005; 96: 1031-1035.

[47] Dhar KK, Branigan K, Parkes J, Howells RE, Hand P, Musgrove C, Strange RC, Fryer AA, Redman CW, Hoban PR. Expression and subcellular localization of cyclin D1 protein in epithelial ovarian tumour cells. Br J Cancer 1999; 81: 1174-1181.