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Forkhead box O-class 1 and Forkhead box G1 as Prognostic Markers for Bladder Cancer

Forkhead box O-class 1 (*FOXO1*) is a key regulator of glucose homeostasis, cell-cycle progression, and apoptosis. Its functions are modulated by forkhead box G1 (*FOXG1*), which acts as a transcriptional repressor with oncogenic potential. Real-time PCR and immunohistochemical staining were performed in 174 primary bladder cancer specimens and 21 normal bladder mucosae to evaluate these genes. *FOXO1* and *FOXG1* mRNA expression in cancer tissues were higher than in normal mucosae (each $P<0.001$). *FOXO1* mRNA levels were significantly higher in samples of non-progressed patients ($P<0.001$), but *FOXG1* were enhanced in those of progressed patients ($P=0.019$). On univariate analysis, *FOXO1* mRNA expression was significantly associated with grade, stage, recurrence, progression and survival (each $P<0.05$). On multivariate analysis, increased *FOXO1* mRNA expression was associated with both reduced disease progression (odds ratio [OR], 0.367; 95% confidence interval [CI], 0.163-0.826, $P=0.015$) and enhanced disease-free survival (OR, 3.262; 95% CI, 1.361-7.820, $P=0.008$). At a median follow-up of 33 months (range 2 to 156), the patients with a high *FOXO1* mRNA expression had a significantly prolonged survival ($P=0.001$). Immunohistochemical findings of *FOXO1* were generally concordant with mRNA expression levels. In conclusion, *FOXO1* may be a promising marker for predicting progression in human bladder cancers.

Key Words : *FOXO1; FOXG1; Urinary Bladder Neoplasms; Prognostic Factor; Real Time PCR; Immunohistochemistry*

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INTRODUCTION

Although epidemiologic and experimental evidences favor a strong role of chemical carcinogens in the etiology of bladder cancer, many cases arise without obvious exposure to known carcinogens (1). It is likely that all malignancies involve aberrations of normal mechanisms regulating cell differentiation and proliferation, often with derangements in the genetic composition of malignant cells. Since mechanisms usually exist in all cells to repair mutated or miscopied DNA or to affect the death of cells containing such altered DNA, the failure of these safeguard mechanisms must occur in most, if not all, malignancies. All of these influences undoubtedly play important roles in determining the development of bladder cancer.

Apoptosis is clearly an advantageous response to DNA damage if DNA repair fails, because it allows multicellular organisms to eliminate potentially harmful cells. Eliminated cells can be replaced from the organism's pool of undamaged cells. Depending on the location, environment, or extent of damage, apoptosis may even be a primary response (2). Therefore, it is not surprising that aberrations in apoptosis can be detrimental and that the failure of dividing cells to initiate apoptosis after sustaining severe DNA damage contributes to cancer (3).

Survival factors suppress the intrinsic cell-death machinery, thereby preventing apoptosis (4). Forkhead box O-class (FOXO) transcription factors, including *FOXO1*, FOXO3a, and FOXO4, function as tumor-suppressor proteins by inhibiting cell proliferation, promoting apoptosis, and protecting cells from oxidative stress and DNA damage. The potency of these functions is tightly regulated by phosphorylation, acetylation, and ubiquitination. *FOXO1* is a key regulator of glucose homeostasis, cell-cycle progression, and apoptosis (5). Emerging evidence indicates that protein levels of *FOXO1* are regulated by phosphoinositide-3-kinase-protein kinaseB (PI3K-PKB/c-Akt)-mediated phosphorylation. Akt is highly active in human cancers due to the loss of phosphatase and tensin homolog (PTEN) (6). *FOXO1* is modulated by forkhead box G1 (*FOXG1*), another member of the Fox transcription factor family, which acts as a transcriptional repressor with oncogenic potential (7). *FOXG1* recognizes some of the same targets as FOXO, but functions as a repressor rather

than an activator (8).

However, little is known about the properties and roles of *F0X01* and *F0XG1* in human bladder cancer. In this study, we explored relationships between these genes and clinicopathologic characteristics in bladder cancer patients using real-time polymerase chain reaction (PCR) and immunohistochemical staining.

MATERIALS AND METHODS

Patients and tissue samples

One hundred and seventy-four primary bladder cancer samples were taken in the Chungbuk National University Hospital, Cheongju, Korea. Histological diagnoses revealed that all patients had transitional cell carcinoma. Table 1 lists demographic data. Twenty one normal bladder tissues were obtained from patients with benign diseases. These were dissected in order to separate from the mucosa from the underlying smooth muscle, which were histologically confirmed normal mucosae on frozen sections. Informed consent was obtained from each subject and the study was approved by the Institutional Review Board of the Chungbuk National University College of Medicine. Median follow-up was 33 months (range 2 to 156). In this study, we defined the superficial recurrence as the cancer recurrence of primary superficial bladder cancer without progression, and the progression as the cancer progression both of superficial bladder cancer to invasive or metastatic disease and of invasive cancer to metastatic disease after adequate treatment. All specimens were rapidly frozen in liquid nitrogen and stored at -80˚C until the RNAs were extracted.

Real-time PCR

Total RNA was isolated from the tissues with TRIzol reagent (Life Technologies, NY, U.S.A.) according to the manufacturer's instructions. cDNA was prepared from 1 μg of total RNA by random priming using a First-Strand cDNA Synthesis Kit (Amersham Biosciences Europe GmbH, Freiburg, Germany) according to the manufacturer's protocol. To quantify the expression levels of *F0X01*, real-time PCR amplification was performed with a Rotor Gene 3000 PCR instrument (Corbett Research, Mortlake, Australia). Quantitative values were obtained from the cycle threshold (Ct) number at which increase in the signal associated with exponential growth of PCR products began to be detected. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was chosen as an endogenous RNA reference gene. Each sample was checked on the basis of its *GAPDH* content. Real-time PCR assays using SYBR Premix EX *Taq* (TAKARA BIO INC.,

Table 1. Clinico-pathological features and mRNA expression levels of *FOXO1* and *FOXG1* in primary bladder transitional cell carcinomas

Variables	No. of patients (%)	*FOXO1* ($\times 10^3$ copies/μL)	P*	*FOXG1* ($\times 10^3$ copies/μL)	P*
Sex					
Male	145 (83.3)				
Female	29 (16.7)				
Age (yr)	64.58±0.89				
Normal versus cancer					
Normal	21	14.57±1.11	<0.001	0.92±0.1	<0.001
Cancer	174	71.17±1.11		2.58±0.4	
Stage					
Superficial	111 (63.8)	93.86±1.14	0.001	2.57±0.42	0.966
Invasive	63 (36.2)	43.71±1.18		2.60±0.61	
Grade					
Low	116 (66.7)	82.44±1.14	0.048	2.08±0.81	0.134
High	58 (33.3)	53.03±1.19		1.81±0.46	
Superficial recurrence					
No recurrence	72 (64.9)	113.92±1.18	0.042	2.53±0.55	0.173
Recurrence	39 (35.1)	65.64±1.23		3.49±0.85	
Progression					
No progression	135 (77.6)	87.38±1.13	<0.001	2.37±0.38	0.019
Progression	39 (22.4)	34.98±1.20		4.84±1.07	
Survival					
Alive	137 (78.7)	87.66±1.13	0.001	2.68±0.42	0.239
Dead	37 (21.3)	34.33±1.19		3.96±0.99	

*Determined using the t-test.
FOXO1, Forkhead box O-class 1; *FOXG1*, Forkhead box G1.

Otsu, Japan) were carried out in micro-reaction tubes (Corbett Research). The PCR reaction was performed in a final volume of 10 μL, consisting of 5 μL of 2 × SYBR Premix EX *Taq* buffer, 0.5 μL of each 5′- and 3′-primer (10 pM/μL), and 1 μL of sample cDNA. To amplify the target and reference genes, the primers were used to amplify: *FOXO1* (153 bp) 5′-atggtcaagagcgtgccc-3′ and 5′-gattgagcatccaccaag-3′; and *FOXG1* (150 bp), 5′-ttcagctacaacgcgctcat-3′ and 5′-acagattgtggcggatggag-3′.

The product was purified with a QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany), quantified with a spectrophotometer (Perkin Elmer MBA2000, Shelton, CT, U.S.A.), and sequenced with an automated laser fluorescence sequencer (ABI PRISM 3100 Genetic Analyzer, Shelton, CT, U.S.A.). The known concentration of the product was 10-fold serially diluted from 4.05×10^5 copies/μL to 4.05×10^2 copies/μL. The dilution series of the PCR products were used to establish the standard curve for the real-time PCR. The real-time PCR conditions were 1 cycle at 96℃ for 20 sec, followed by 40 cycles of 2 sec at 96℃, 20 sec at 60℃, and 20 sec at 72℃. The melting program was performed at 72-95℃ with a heating rate of 1℃ per 45 sec. Spectral data were captured and analyzed using Rotor-Gene Real-Time Analysis Software 6.0 Build 14 (Corbett Research, Mortlake, Australia).

Immunohistochemical staining

Immunohistochemical staining was performed in matched 174 archival bladder tissue paraffin blocks. All cases were retrospectively identified from the surgical pathology files of the same hospital and the corresponding slides were reviewed to reconfirm the pathological parameters including grade and stage. All archival materials were routinely fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections (4 μm) were prepared on silane-coated slides (Sigma, St. Louis, MO, U.S.A.). A DakoCytomation Immunostaining Kit (Glostrup, Denmark) was used. Tissue sections on microslides were deparaffinized with xylene, hydrated in serially-diluted alcohol, and immersed in 3% H_2O_2 to quench endogenous peroxidase activity. For antigen retrieval, the slides were treated with microwaves in 10 mM borate buffer (pH 8.0) for 15 min. The sections were then incubated with primary antibodies (Santa Cruz Biotechnology, Inc. Santa Cruz, CA, U.S.A.) for 60 min, rinsed three times with washing buffer, and further incubated for 20 min with an Envision detection system (anti-rabbit; DakoCytomation). After rinsing, immunostaining was performed for 5 min with liquid 3,3′-diaminobenzidine (DiNonA, Seoul, Korea). The sections were then counterstained with Meyer's hematoxylin, dehydrated, and mounted with Canada balsam. The rinsing solution was distilled water with 0.1% TWEEN 20. Three independent investigators evaluated the immunohistochemical staining without knowledge of the clinicopathologic parameters. *FOXO1* staining was mainly cytoplasmic. As cognate anti-*FOXG1* antibody was not commercially available, we were unable to study this factor immunohistochemically.

For semiquantitative assessment of the immunohistochemical results, the mean percentage of positive tumor cells was determined in at least 10 random fields at ×400 magnification in each section. It was graded as focal (10%), regional (11-50%), or diffuse (>50%). The intensity of immunoreaction was graded weak, moderate, or intense. The mean percentage of positive tumor cells and the staining intensity were then combined to produce immunohistochemical results. The results were graded as negative (0), moderate regional (1), moderate diffuse (2), intense regional (3), and intense diffuse (4). Inter-observer differences were minimal. The consensus opinions were used to assign final scores to the disputed cases before data analysis.

Statistical analysis

All statistical analyses were carried out using the SPSS package, Release 10.0 (SPSS Inc., Chicago, IL, U.S.A.). Because of its highly skewed distribution, the *FOXO1* and *FOXG1* mRNA data were examined as the natural log function and subsequently back transformed for the interpretation of the model results. The mRNA data were presented as the means and SEMs. Student's t-tests were applied to assess the association of the mRNA expression levels with the development, progression, recurrence of the cancer and with survival. Pearson's correlation was used for the relation between the expression level of *FOXO1* and *FOXG1*. Multivariate logistic regression analyses were also performed to identify the factors that had a significant effect on cancer progression and survival. We used receiver-operating-characteristic (ROC) curves to analyze *FOXO1* mRNA levels in order to determine the cutoff point (51.48×10^3 copies/μL) that yielded the highest combined sensitivity and specificity for disease-free survival. Kaplan-Meier curves were generated and compared by the log rank test for survival rates. The immunohistochemical data were analyzed using chi-square method.

RESULTS

Quantification of *FOXO1* and *FOXG1* mRNA expression levels

Considerably higher *FOXO1* and *FOXG1* expression were observed in bladder cancer tissues than in normal mucosae (each $P<0.001$). Table 1 summarizes the expression levels of *FOXO1* and *FOXG1* according to the stage, grade, superficial recurrence, progression, and disease-free survival of bladder cancer. The *FOXO1* expression levels in superficial bladder cancers were significantly higher than in invasive cancers ($P<0.001$). *FOXO1* expression levels were significantly ele-

vated in low-grade compared with high-grade bladder cancers (P=0.048). *FOXG1* did not show any relations with the stage and grade of bladder cancer (each P>0.05).

In the superficial bladder cancers, the expression levels of *FOXO1* were enhanced in non-recurred patients compared with recurred patients (P=0.042), whereas *FOXG1* did not. The *FOXO1* mRNA levels were significantly higher in bladder cancer tissues from the patients without progression than in progressed cases (P<0.001). In contrast, the expression of *FOXG1* mRNA in primary bladder cancer that had progressed was enhanced compared to cases that had not (P=0.019). In addition, a weak negative correlation between mRNA expression levels of *FOXO1* and *FOXG1* was observed in bladder cancer tissues (Pearson's sample correlation coefficient r=-0.220, P=0.008).

The association between the *FOXO1* expression levels and the disease-free survival of patients was also analyzed. Our data showed that the patients with elevated *FOXO1* mRNA expression in their primary bladder cancers had significant survival benefits compared to those with low expression. Expression levels of *FOXO1* in primary cancer tissues of living patients were significantly higher than in those of deceased cases (P<0.001).

Multivariate analysis results showed that the cancer stage and *FOXO1* mRNA expression level were strong predictors of cancer progression and disease-free survival (Table 2). In particular, increased *FOXO1* mRNA expression levels were associated with both reduced disease progression (odds ratio [OR], 0.367; 95% confidence interval [CI], 0.163-0.826, P=0.015) and enhanced disease-free survival (OR, 3.262; 95% CI, 1.361-7.820, P=0.008).

Fig. 1 shows the Kaplan-Meier analyses of bladder cancer disease-free survival stratified by *FOXO1* mRNA expression level. The patients with elevated *FOXO1* mRNA expression level in their primary bladder cancers had significant survival benefit compared to those with low-expression (P=0.001).

Table 2. Multivariate logistic regression analyses of age, sex, grade, stage, and *FOXO1* mRNA expression levels in bladder cancers for the prediction of bladder cancer progression and disease-free survival

Variables	Odds ratio	95% CI	*P*
Prediction of progression			
Age	1.198	0.536-2.678	0.660
Sex	1.964	0.766-5.032	0.160
Grade	0.904	0.336-2.435	0.842
Stage	4.308	1.598-11.615	0.004
mRNA expression levels of *FOXO1*	0.367	0.163-0.826	0.015
Prediction of disease-free survival			
Age	0.326	0.271-1.543	0.326
Sex	0.613	0.223-1.685	0.343
Grade	1.496	0.523-4.280	0.452
Stage	0.111	0.037-0.329	<0.001
mRNA expression levels of *FOXO1*	3.262	1.361-7.820	0.008

FOXO1, Forkhead box O-class 1; 95% CI, 95% confidence interval.

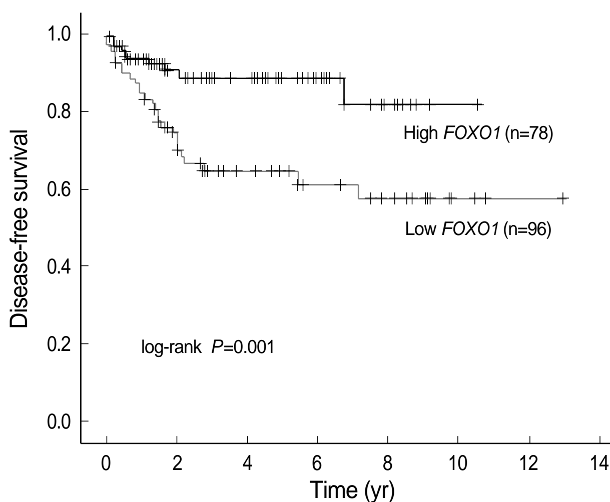

Fig. 1. Disease-free survival and *FOXO1* mRNA expression levels. The cut-off point (51.48×10^3 copies/μL) with the highest combined sensitivity and specificity was determined on ROC curve.

Expression of *FOXO1* by immunohistochemical staining

Immunohistochemistry revealed cytoplasmic staining of *FOXO1* in urinary bladder cancers (Fig. 2). Table 3 shows the relationship between expression levels of *FOXO1* and bladder cancer. *FOXO1* expression levels were significantly higher in low-grade cancers than in high-grade cancers (P<0.001). *FOXO1* was more strongly expressed in superficial bladder

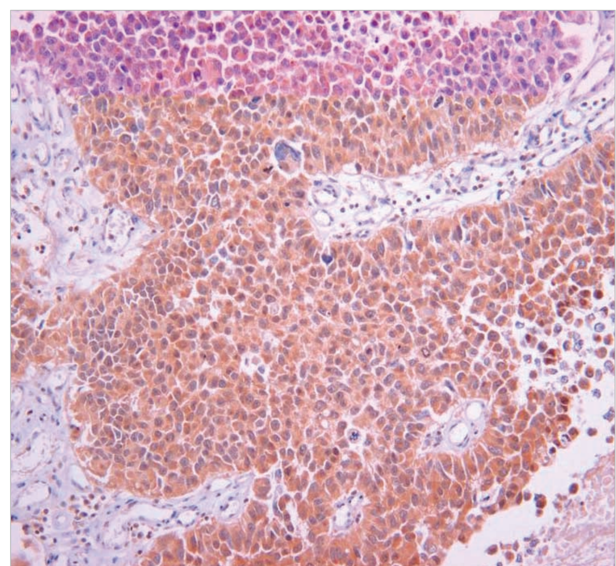

Fig. 2. The *FOXO1* expression shows strong cytoplasmic reactivity on urothelial cancer cells (×200).

Table 3. Clinico-pathological features and *FOXO1* immunohistochemical staining scores of primary bladder transitional cell carcinomas

Variables	Immunohistochemical staining score (%)†					P*
	0	1	2	3	4	
Stage						
Superficial (n=111)	22 (19.8)	20 (18.0)	47 (42.3)	18 (16.2)	4 (3.6)	<0.001
Invasive (n=63)	38 (60.3)	19 (30.2)	4 (6.3)	1 (1.6)	1 (1.6)	
Grade						
Low (n=116)	24 (20.7)	24 (20.7)	46 (39.7)	18 (15.5)	4 (3.4)	<0.001
High (n=58)	36 (62.1)	15 (25.9)	5 (8.6)	1 (1.7)	1 (1.7)	
Superficial recurrence						
No recurrence (n=72)	18 (25.0)	15 (20.8)	30 (41.7)	8 (11.1)	1 (1.4)	<0.003
Recurrence (n=39)	4 (10.3)	5 (12.8)	17 (43.6)	10 (25.6)	3 (7.7)	
Progression						
No progression (n=135)	40 (29.6)	24 (17.8)	50 (37.0)	17 (12.6)	4 (3.0)	<0.001
Progression (n=39)	20 (51.3)	15 (38.5)	1 (2.6)	2 (5.1)	1 (2.6)	
Survival						
Alive (n=137)	39 (28.5)	26 (19.0)	50 (36.5)	17 (12.4)	5 (3.6)	<0.001
Dead (n=37)	21 (56.8)	13 (35.1)	1 (2.7)	2 (5.4)	0 (0)	

*Test for a linear trend across the immunohistochemical staining score; †0 for negative, 1 for moderate regional, 2 for moderate diffuse, 3 for intense regional, 4 for intense diffuse.
FOXO1, Forkhead box O-class 1.

cancers than in invasive cancers (P<0.001). Although *FOXO1* expression was higher in patients with recurrence than in those without recurrence, the difference was not statistically significant (P=0.143). *FOXO1* expression in bladder cancer tissues from non-progressed patients was significantly higher than in those from progressed cases (P=0.005). A significant correlation was also found between *FOXO1* expression levels and disease-free survival (P=0.003).

DISCUSSION

The FOXO family is regulated by the PI3K-PKB/c-Akt pathway. PKB-induced phosphorylation inhibits transcriptional activity of the FOXO members, which control the cell cycle, cell death, cell metabolism, and response to oxidative stress (9). Studies in mammalian cells have shown that the overproduction of *FOXO1* induces either cell-cycle arrest or apoptosis (9). By increasing the production of the cyclin-dependent kinase inhibitor p27*kip1*, FOXO transcription factors cause cell-cycle arrest in the G1 phase of the cell cycle (10). Recently, FOXO factors have also been implicated in the control of proper progression through the G2-M phase of the cell cycle (11). The production of FOXO family members can also cause cell death by apoptosis (12).

Support for a connection between FOXO proteins and oncogenic transformation is provided by another Fox family protein, *FOXG1*. This transcription factor was originally identified as brain factor 1 and has also been recovered as the retroviral oncoprotein Qin in ASV31 (13, 14). The oncogenic transformation induced by retroviral expression of *FOXG1* directly correlates to transcriptional repression (7, 15). In addition, *FOXG1* binds the consensus sequence tgtaaacaaa (15), which is similar to the binding motif for FOXO proteins (gtaaacaa) (16). Using cotransfections of *FOXO1* and *FOXG1* with the reporter pGL3-CMV-3xIRS, Aoki et al. demonstrated that *FOXG1* is a strong inhibitor of *FOXO1*-mediated transcriptional activation. They suggested that *FOXG1* and *FOXO1* might negatively and positively regulate a shared suite of target genes, respectively (17).

In this study, we investigated the mRNA expression levels of *FOXO1* and *FOXG1* in human bladder cancer. This is the first study to investigate the relationships between the expression of these genes and clinico-pathological parameters in bladder cancer to our knowledge. Our data shows that *FOXO1* and *FOXG1* are more highly expressed in bladder cancer tissues than in normal bladder mucosae, which suggests that *FOXO1* might be activated during tumorigenesis in the bladder. At present, the exact mechanisms for enhanced expression of *FOXO1* and *FOXG1* in bladder cancer are unknown. One possible explanation is based on the relationship between *FOXO1* and *FOXG1*. The function of *FOXO1* is further modulated by *FOXG1*. *FOXG1* acts as a transcriptional repressor, has oncogenic potential (7), and can repress some of the same targets that are activated by *FOXO1* (8). Therefore, *FOXO1* expression might increase to compete with *FOXG1* suppression. Another possible explanation for enhanced *FOXO1* expression may be that, a variety of much stronger oncogenic activity mask the protective effect of *FOXO1*.

We also analyzed the expression levels of *FOXO1* in relation to the clinical findings in bladder cancer patients. *FOXO1* immunohistochemical findings were generally in concordance with mRNA expression levels, suggesting that *FOXO1* expression might be primarily determined at the transcription-

al level.

Our data demonstrate that *FOXO1* expression is enhanced in superficial bladder cancer compared to invasive cancer and that *FOXO1* expression levels are higher in low-grade cancers (grades 1 and 2) compared to high-grade cancers (grade 3). Bladder cancers with increased *FOXO1* expression exhibited pathological features of less aggressive, superficial and low-grade bladder cancers. There is a clear survival advantage for the advanced cancer cell that can protect itself from apoptosis. However, a rapidly growing, infiltrative, advanced tumor that is outgrowing its blood supply and mutating its DNA may have enhanced activation of an apoptosis-related pathway despite protective mechanisms acquired by the tumor cells. However, it remains unclear why *FOXO1* expression increases in superficial cancers as compared to invasive tumors.

Although the immunohistochemical findings were not statistically significant, the expression levels of *FOXO1* mRNA in primary superficial bladder cancer tissues were significantly higher in non-recurred than in recurred patients in our study. When comparing primary bladder cancer cases that had progressed with those that had not, we observed significantly higher expression of *FOXO1* in the latter group and higher levels of *FOXG1* in the former. We observed a negative correlation between the levels of *FOXO1* and *FOXG1* mRNA expression in bladder cancer tissue, a finding supported by Aoki et al., who demonstrated that *FOXG1* strongly inhibited *FOXO1*-mediated transcriptional activation (17). These results indicate that *FOXO1* and *FOXG1* expression may be useful prognostic markers for bladder cancer progression. Patients with enhanced *FOXO1* expression exhibited a higher disease-free survival rate than those with low *FOXO1* expression, implying that enhanced *FOXO1* expression might suppress disease progression and provide a survival benefit for bladder cancer patients. These findings are further supported by the multivariate analysis, which showed that the cancer stage and *FOXO1* mRNA expression level were strong predictors of cancer progression and disease-free survival.

In conclusion, enhanced expression of *FOXO1* and *FOXG1* are strongly associated with bladder cancer development. Moreover, enhanced expression of *FOXO1* is positively associated with low rate of recurrence or progression and with survival, whereas increased expression of *FOXG1* correlates to disease progression. Thus these genes may be useful prognostic markers for human bladder cancers.

REFERENCES

1. Messing EM. *Urothelial tumors of the bladder. In: Wein AJ, Kavoussi LR, Novick AC, Partin AW, Peters CA, eds, Campbell-Walsh urology. 9th ed. Philadelphia: Saunders, 2007; 2407-46.*
2. Evan G, Littlewood T. *A matter of life and cell death. Science 1998; 281: 1317-22.*
3. Ashkenazi A, Dixit VM. *Death receptors: signaling and modulation. Science 1998; 281: 1305-8.*
4. Jacobson MD, Weil M, Raff MC. *Programmed cell death in animal development. Cell 1997; 88: 347-54.*
5. Modur V, Nagarajan R, Evers BM, Milbrandt J. *FOXO proteins regulate tumor necrosis factor-related apoptosis inducing ligand expression. Implications for PTEN mutation in prostate cancer. J Biol Chem 2002; 277: 47928-37.*
6. Matsuzaki H, Daitoku H, Hatta M, Tanaka K, Fukamizu A. *Insulin-induced phosphorylation of FKHR (Foxo1) targets to proteasomal degradation. Proc Natl Acad Sci USA 2003; 100: 11285-90.*
7. Li J, Thurm H, Chang HW, Iacovoni JS, Vogt PK. *Oncogenic transformation induced by the Qin protein is correlated with transcriptional repression. Proc Natl Acad Sci USA 1997; 94: 10885-8.*
8. Seoane J, Le HV, Shen L, Anderson SA, Massague J. *Integration of Smad and forkhead pathways in the control of neuroepithelial and glioblastoma cell proliferation. Cell 2004; 117: 211-23.*
9. Burgering BM, Kops GJ. *Cell cycle and death control: long live Forkheads. Trends Biochem Sci 2002; 27: 352-60.*
10. Medema RH, Kops GJ, Bos JL, Burgering BM. *AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1. Nature 2000; 404: 782-7.*
11. Alvarez B, Martinez AC, Burgering BM, Carrera AC. *Forkhead transcription factors contribute to execution of the mitotic programme in mammals. Nature 2001; 413: 744-7.*
12. Nakamura N, Ramaswamy S, Vazquez F, Signoretti S, Loda M, Sellers WR. *Forkhead transcription factors are critical effectors of cell death and cell cycle arrest downstream of PTEN. Mol Cell Biol 2000; 20: 8969-82.*
13. Li J, Vogt PK. *The retroviral oncogene qin belongs to the transcription factor family that includes the homeotic gene fork head. Proc Natl Acad Sci USA 1993; 90: 4490-4.*
14. Tao W, Lai E. *Telencephalon-restricted expression of BF-1, a new member of the HNF-3/fork head gene family, in the developing rat brain. Neuron 1992; 8: 957-66.*
15. Li J, Chang HW, Lai E, Parker EJ, Vogt PK. *The oncogene qin codes for a transcriptional repressor. Cancer Res 1995; 55: 5540-4.*
16. Furuyama T, Nakazawa T, Nakano I, Mori N. *Identification of the differential distribution patterns of mRNAs and consensus binding sequences for mouse DAF-16 homologues. Biochem J 2000; 349: 629-34.*
17. Aoki M, Jiang H, Vogt PK. *Proteasomal degradation of the FoxO1 transcriptional regulator in cells transformed by the P3k and Akt oncoproteins. Proc Natl Acad Sci USA 2004; 101: 13613-7.*