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Original Article

Association of *GATA5* methylation with clinocopathological characteristics in non-muscle invasive bladder cancer

Hyung-Yoon Yoon[1], Sung Pil Seo[2], Sang Keun Lee[2], Ho Won Kang[2], Won Tae Kim[2], Dong Hee Ryu[3], Seok-Joong Yun[2], Sang-Cheol Lee[2], Wun-Jae Kim[2], Yong-June Kim[2]*

[1]*R&D center, Advanced Technology,Cheongju 28413, Korea*
[2]*Department of Urology, College of Medicine, Chungbuk National University, Cheongju 28644, Korea*
[3]*Department of Surgery, College of Medicine, Chungbuk National University, Cheongju 28644, Korea*

DNA methylation is the most common and well-characterized epigenetic change in human cancer. Recently, the association between *GATA-binding protein 5 (GATA5)* methylation and carcinogenesis of various types of tumors was investigated. The aim of the present study was to evaluate the effect of *GATA5* methylation status on clinicopathological features and prognosis in primary non-muscle invasive bladder cancer (NMIBC) patients with a long-term follow-up period. The *GATA5* methylation status was determined for 171 human bladder specimens (eight normal controls [NCs] and 163 primary NMIBC patients) using quantitative pyrosequencing analysis. The primary NMIBC tissues were obtained from patients who underwent transurethral resection (TUR) for histologically diagnosed transitional cell carcinomas between 1995 and 2012 at Chungbuk National University Hospital. *GATA5* methylation was significantly higher in NMIBC patients than in NCs and was significantly associated with higher grade and more advanced stage of cancer. Kaplan-Meier estimates showed significant differences in tumor recurrence and progression according to *GATA5* methylation status (each $p<0.05$). Our results show that increased methylation of *GATA5* was significantly associated with not only aggressive characteristics but also poor prognosis in primary NMIBC patients. Alteration of *GATA5* methylation might be used as a biomarker for prognosis of NMIBC patients. However, prospective and functional investigations are necessary to clarify the role of *GATA5* methylation in future clinical management of patients with NMIBC.

Key words: *GATA5*, methylation, recurrence, progression, urinary bladder neoplasms

Introduction

Bladder cancer (BC) is a heterogeneous disease, which means that pathologically similar tumors may behave differently. In approximately 70% of all BC cases, patients present with non-muscle invasive bladder cancer (NMIBC), whereas the remaining 30% present with muscle invasive bladder cancer (MIBC). The standard treatment for NMIBC is transurethral resection (TUR) complemented by intravesical immunotherapy or chemotherapy to prevent recurrence and progression [1,2]. Numerous factors are likely involved in disease outcome, and many patients with NMIBC experience disease recurrence and progression after primary treatment [1,2]. Therefore, identifying patients at high risk of recurrence and progression who would benefit from more aggressive treatment, as well as those at low risk who require less intensive surveillance after initial adequate therapy, is challenging. Currently, conventional clinicopathological factors are insufficient to predict the outcome of patients with NMIBC. Thus, additional biomarkers are needed for prognosis of NMIBC patients.

Recent advances in our understanding of epigenetic modifications, including DNA methylation, histone modifications, and microRNAs, have provided new opportunities for detecting, treating, and preventing cancer. The usage of DNA methylation as a biomarker has attracted attention in recent years since aberrant DNA methylation is a major characteristic of BC and plays a crucial role in tumor initiation and progression [3-6]. DNA methylation, which inactivates tumor suppressor genes, is the most common and well-characterized epigenetic change in human cancer and may be a potential biomarker for cancer [7,8]. Previous studies have found that *GATA-binding protein 5* (*GATA5)* hypermethylation and associated epigenetic silencing may be involved in carcinogenesis of various tumors such as BC, renal cell carcinoma, and

***Corresponding author:** Yong-June Kim
Department of Urology, College of Medicine, Chungbuk National University, *Cheongju 28644, Korea*
Tel: +82-43-269-6134, Fax: +82-43-269-6144, E-mail: urokyj@gmail.com

gastric, colorectal, and ovarian cancers and are related with tumor aggressiveness and patient prognosis [9-17].

To the best of our knowledge, relatively few studies have evaluated the association between *GATA5* methylation status and BC [16,17]. The aim of the present study was to evaluate the effect of *GATA5* methylation status on clinicopathological features and prognosis in primary NMIBC patients with a long-term follow-up period.

Materials and Methods

Subjects and sample collection

A total of 171 human bladder tissues (eight normal controls [NCs] and 163 NMIBCs) were used for pyrosequencing (PSQ) analyses (Table 1). Primary NMIBC tissues were obtained from patients who underwent TUR for histologically diagnosed transitional cell carcinomas between 1995 and 2012 at Chungbuk National University Hospital. To exclude the possibility of incomplete resection or factors that may affect analyses, patients who were followed for less than 6 months or those that experienced disease relapse within 6 months were excluded from this study. NC tissues were obtained from individuals with benign prostate hyperplasia or bladder injury.

All tumors were macro-dissected within 15 min of surgical resection. Each NMIBC specimen was confirmed by pathological analysis of a tissue section that was obtained from the TUR specimens, immediately frozen in liquid nitrogen, and stored at − 80°C. The specimens were provided by Chungbuk National University Hospital, a member of the National Biobank of Korea, which is supported by the Ministry of Health, Welfare, and Family Affairs. Collection and analysis of all samples were approved by the Chungbuk National University Hospital Institutional Review Board (GR2010-12-010), and informed consent was obtained from each subject.

Tumor staging was classified according to the 2002 TNM classification and 1973 World Health Organization grading systems [18]. Patients with intermediate- or high-risk NMIBC received one cycle of intravesical instillation therapy. Each patient was followed up and managed according to standard recommendations [1,2]. Recurrence was defined as the return of primary NMIBC at a lower or equivalent pathologic stage (Ta/T1), and progression was defined as muscular invasion (TNM stage T2 or higher) or nodal/distant metastatic disease.

DNA extraction and PSQ analysis

Genomic DNA was extracted by standard methods using the Wizard Genomic DNA Purification System (Promega, Madison, WI, USA). Bisulfite conversion of genomic DNA was carried out using an EZ DNA Methylation Kit (Zymo Research, Irvine, CA, USA). DNA methylation status of *GATA5* was assessed by PSQ using PyroMark Q96 ID (Qiagen, Valencia, CA, USA). Primer sequences were as follows: forward primer: TGTGGTAGTTGGTGTAGTAGAG, reverse primer: (Biotin)-AATCTCCCTCCCCCCCACAATC, sequencing primer: GTTGGTGTAGTAGAGG, sequence to analyze: TYGGYGYGGYGGGAYGAGGATTGTGGGGGT. The PCR reaction mixture contained 0.01 μM primers, Bioneer Taq (Bioneer, Daejeon, Korea), and 20 ng of bisulfite-treated DNA.

Thermocycling parameters were as follows: denaturation at 94°C for 5 min, followed by 45 cycles of 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 30 sec, and a final extension at 72°C for 5 min. A biotin-labeled primer was used to purify the final PCR product using streptavidin-coated Sepharose beads (GE Healthcare, Wauwatosa, Wisconsin, USA). The PCR product was bound to Sepharose beads, purified, washed, denatured using 0.2 M NaOH solution, and washed again. Subsequently, 0.3 μM PSQ sequencing primer was annealed to the purified single-stranded PCR product, and PSQ was performed on a PyroMark Q96 ID (Qiagen, Va-

Table 1. Baseline characteristics of subjects

Variables		NMIBC (n=163)
Age, yrs (mean)		63.2 ± 13.8
Gender, no. of patients (%)	Male	133 (81.6)
	Female	30 (18.4)
No. of tumors (%)	Single	96 (58.9)
	Multiple	67 (41.1)
Tumor size (%)	<3 cm	89 (54.6)
	≥3 cm	74 (45.4)
Grade, no. of patients (%)	G1	53 (32.5)
	G2	86 (52.8)
	G3	24 (14.7)
T Stage	Ta	61 (37.4)
	T1	102 (62.6)
Intravesical treatment, no. of patients (%)	No	83 (50.9)
	Yes	80 (49.1)
Recurrence-free survival, months (median)		43.9 (6.1~171.5)
Recurrence, no. of patients (%)	No	107 (65.6)
	Yes	56 (34.4)
Progression free survival, months (median)		52.3 (6.6~164.9)
Progression, no. of patients (%)	No	145 (89.0)
	Yes	18 (11.0)

NMIBC, non-muscle invasive bladder cancer.

lencia, CA, USA). Target CpG sites were evaluated using the instrument software (PSQ96MA 2.1, Qiagen, Valencia, CA, USA), which converts pyrograms to numerical values for peak heights and calculates the proportion of methylation at each base as a C/T ratio. Data analysis was performed using PyroMark Q96 ID Software v.1.0 software (Qiagen, Valencia, CA, USA).

Statistical analysis

Differences in *GATA5* methylation values between groups were assessed using a two-sample t-test or ANOVA trend analyses using polynomial contrasts. Median values were used as a cut-off point to divide patients into subgroups (hypomethylation or hypermethylation), and survival functions of *GATA5* genes were evaluated. The Kaplan-Meier curves were used to estimate time to recurrence or progression according to methylation status, and differences were evaluated using log-rank tests. Using multivariate Cox proportional hazards regression analyses, the prognostic value (recurrence or progression) of methylation status was evaluated and adjusted for well-known clinicopathological factors (sex, age, tumor size, tumor number, intravesical therapy, grade, and stage). Statistical analysis was performed using SPSS 20.0 software (IBM, Armonk, NY, USA). A *p*-value <0.05 was considered statistically significant.

Table 2. Association between *GATA5* methylation and clinicopathological characteristics

Variables		Methylation level (%)	*p*-value
Normal *vs*. cancer	Normal	17.6 ± 3.6	<0.001[1)]
	Cancer	53.6 ± 24.7	
Number of tumors	Single	50.9 ± 26.1	0.081[1)]
	Multiple	57.5 ± 22.0	
Tumor size	<3 cm	51.2 ± 25.5	0.236[1)]
	≥3 cm	56.1 ± 24.0	
Grade	G1	46.4 ± 24.7	0.003[2)]
	G2	54.3 ± 23.7	
	G3	67.0 ± 23.3	
T stage	Ta	44.8 ± 26.0	0.001[1)]
	T1	58.9 ± 22.4	
Recurrence	No	43.6 ± 23.3	0.002[1)]
	Yes	57.6 ± 24.6	
Progression	No	51.7 ± 24.1	0.012[1)]
	Yes	68.7 ± 24.7	

[1)]*P*-value calculated using Student's t-test.
[2)]*P*-value calculated using ANOVA trend analyses test.
GATA5, GATA binding protein 5.

Results

Characteristics of study subjects

Baseline characteristics of NC and NMIBC patients are presented in Table 1. Mean age was 63.2 ± 13.8 years for patients with NMIBC. Mean recurrence- and progression-free survival times were 47.3 ± 40.1 months (median, 35.9; range, 6.1 to 171.5) and 62.3 ± 41.7 months (median, 52.3; range, 6.6 to 171.5), respectively.

Relationship between methylation levels and clinicopathological variables

As shown in Table 2, methylation levels of *GATA5* were significantly higher in samples from NMIBC patients (53.6 ± 24.7%) than in those from NC patients (17.6 ± 3.6%) ($p<0.001$). To evaluate the relationship between methylation patterns and clinicopathological factors, methylation levels were compared with well-known prognostic factors such as tumor number, size, grade, and stage. High levels of *GATA5* methylation were significantly associated with higher grade and more advanced stage tumors (each $p<0.001$).

Methylation status as a predictor of prognosis

To evaluate the effect of *GATA5* status on prognosis (recurrence or progression), we compared *GATA5* methylation level based on prognosis. The *GATA5* methylation level was significantly higher in the poor prognosis group (recurrence or progression) compared to the favorable prognosis group (Table 2). To further determine the correlation between methylation and prognosis, *GATA5* methylation levels of each patient were dichotomized (hypomethylation or hypermethylation) with the median defined as the cut-off point. Kaplan-Meier estimates revealed that the *GATA5* hypermethylation group had significantly less time to recurrence and progression than the *GATA5* hypomethylation group (Fig. 1, log-rank test, each $p<0.05$). Univariate Cox regression analyses showed that *GATA5* methylation status was a predictive factor of recurrence (hazard ratio [HR], 1.908; 95% confidence interval [CI], 1.110~3.279; $p=0.019$) and progression (HR, 3.725; 95% CI, 1.213~11.439; $p=0.022$) in patients with primary NMIBC. Upon multivariate Cox regression analyses, *GATA5* methylation status served as an independent predictive factor of recurrence (HR, 1.470; 95% CI, 0.818~2.643; $p=0.128$) and progression (HR, 2.554; 95% CI, 0.758~8.603; $p=0.130$) in patients with primary NMIBC, although it did not reach statistical significance.

Discussion

Our results show that methylation level of *GATA5* was significantly higher in tissues from NMIBC patients com-

pared to NC patients, and hypermethylation of *GATA5* was significantly associated with higher tumor grade and advanced pathological stage. Although *GATA5* methylation status was not an independent prognostic indicator of recurrence and progression, it was significantly associated with reduced time to recurrence and progression in primary NMIBC patients.

Genetics refers to the study of information inherited on the basis of gene sequences, whereas epigenetics is the study of reversible and inheritable changes in gene function, or of other cell phenotypes without alteration of DNA sequences. DNA methylation occurs throughout the genome and involves the addition of a methyl group to the cytosine ring of the CpG dinucleotide [3]. The methylation pattern is established during development and is normally maintained throughout the life of an individual. Thus, DNA methylation is a key regulator of gene transcription and genomic stability, and inappropriately altered DNA methylation patterns are frequently detected as epigenetic changes in human cancers. In BC, hypermethylation of tumor suppressor genes such as *APC, ARF, CDH1, GSTP1, MGMT, CDKN2A, RARβ2, RASSF1A, TIMP3*, and *RUNX3* has been reported [4-6]. As promoter hypermethylation is frequently observed in BC, several authors have investigated its occurrence in exfoliated urinary cells or tumor tissues. Methylation of these genes may facilitate cancer detection and correlate with a poor prognosis [4-6]. Using survival as the end point, different studies have demonstrated that methylation of *CDH1, FHIT, LAMC2, RASSF1A, TIMP3, SFRP1, SOX9, PMF1, RUNX3*, and *SYNPO2* is associated with poor survival in patients with MIBC [6]. Thus, markers for aberrant methylation may be a potential gateway for monitoring and determining prognosis of BC. In the present study, *GATA5* hypermethylation was associated with aggressive tumor features and poor prognosis. Although *GATA5* methylation status was not an independent prognostic indicator in multivariate analysis, the results suggest the possibility of *GATA5* gene as a methylation-based biomarker in BC.

GATA transcription factors containing two conserved zinc finger DNA-binding domains recognizing the WGATAR sequence (W=A or T and R=A or G) play a critical role in regulating embryonic morphogenesis and cellular differentiation [19]. There exist six GATA factors divided into two groups, *GATA1/2/3* and *GATA4/5/6*, according to tissue-specific expression pattern. *GATA1*, *GATA2*, and *GATA3* of the GATA transcription factor family are involved in cellular lineage and hematopoietic development, whereas *GATA4*, *GATA5*, and *GATA6* are involved in epithelial and endodermal differentiation [20,21]. *GATA6* may function as an oncogene since it is often up-regulated in proliferating progenitor cells [22]. In contrast, *GATA4* and *GATA5* may function as tumor suppressors since they are potential up-regulators of differentiation-related genes in endoderm-derived organs [23]. In addition, allelic imbalances in chromosomal loci for *GATA4* 8p23.1-p22 and *GATA5* 20q13.2-q13.3 are frequent areas of chromosomal deletion in neoplasms [24]. Epigenetic alteration of *GATA5* has been detected in various types of cancers and is closely related with tumor characteristics [9-15]. Recently, Peters *et al*. reported an association between *GATA5* hypermethylation as well as metastasis and progression-free survival in patients with renal cell carcinoma [11]. Only a few reports have detailed the association between *GATA5* methylation and BC [16,17]. A previous study on the methylation status of tumor suppressor genes and its utility for predicting BCG responses in 91 patients with T1G3 high-risk BC reported *GATA5* gene as a novel methylation marker in BC [16]. Moreover, in their study, recurrence and progression rate were significantly associated with *GATA5* methylation status. In another study, methylation status of *GATA5* was used to classify pTa versus pT1 tumors as well as distinguish low grade versus high grade tumors [17]. In accordance with these previous studies, our re-

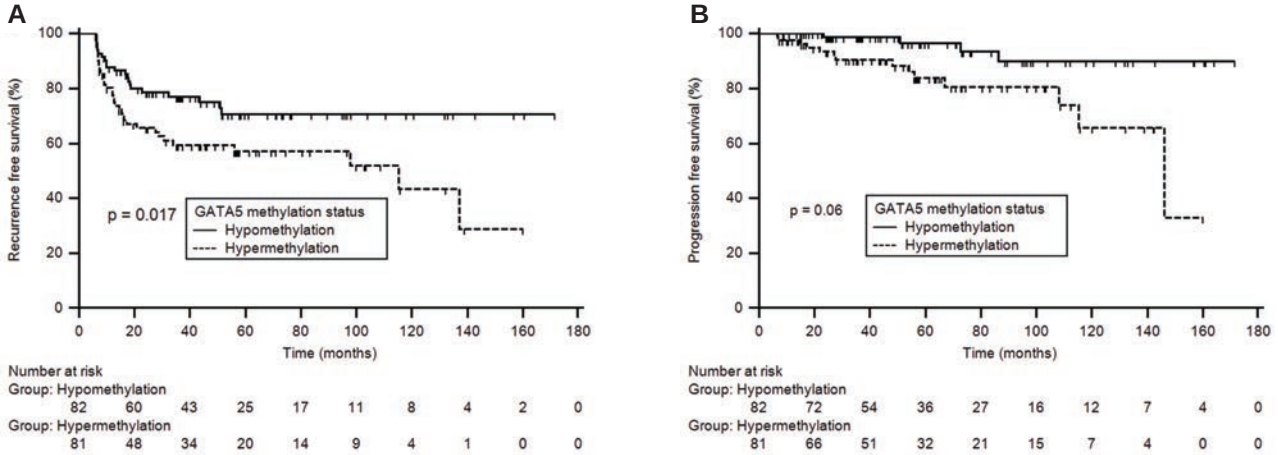

Fig. 1. Kaplan-Meier curves of recurrence (A) and progression (B) according to methylation status of *GATA5*.

sults show that *GATA5* methylation status was associated with aggressive pathological features (stage and grade) and poor prognosis (recurrence and prognosis) in patients with NMIBC [16,17].

A precise causal relationship between *GATA5* hypermethylation and NMIBC has not yet been established. Additionally, even though *GATA5* hypermethylation may have functional significance in BC, it may not play a crucial role in bladder tumor initiation or progression. Although we could not demonstrate the association between *GATA5* hypermethylation and BC, it will be investigated in a further study. The objective of the present study was to identify methylation markers related to NMIBC. Thus, we focused on the association between specific methylation markers and disease phenotype rather than the effects of methylation status on gene transcription and function. The strength of our study was that we performed definitive subgroup analysis after selecting only primary NMIBC patients. As BC is a heterogeneous disease with a prognosis affected by many factors, it is important to evaluate useful genes as prognostic markers within a homogenous study population. Although *GATA5* hypermethylation was not an independent predictor of disease outcome, the results presented herein are promising since clinical significance was evaluated in a relatively large number of human tissue samples obtained from primary NMIBC patients with a long-term follow-up period (median, 52.3; range, 6.6 to 171.5). Therefore, *GATA5* hypermethylation might be serve as a biomarker for tumor prognosis, although further prospective and functional investigations will be necessary to reduce false prediction rates and ensure reliability. Therefore, not only large-scale validation studies of human samples but also functional analysis and gene ontologic evaluation of *GATA5* genes should be performed to gain more insights into their biological mechanisms and clinical relevance.

Our results show that increased methylation of *GATA5* was significantly associated with not only aggressive characteristics but also poor prognosis in patients with primary NMIBC. Alteration of *GATA5* methylation might be used as a biomarker for prediction of prognosis in patients with NMIBC. However, prospective and functional investigations are necessary to clarify the role of *GATA5* methylation in future clinical management of patients with NMIBC.

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ORCID

Yong-June Kim, http://orcid.org/0000-0002-7021-2450

References

1. Babjuk M, Burger M, Zigeuner R, Shariat SF, van Rhijn BW, Comperat E, Sylvester RJ, Kaasinen E, Bohle A, Palou Redorta J, Roupret M, European Association of U. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2013. European Urology 2013;64:639-653.
2. Kamat AM, Hegarty PK, Gee JR, Clark PE, Svatek RS, Hegarty N, Shariat SF, Xylinas E, Schmitz-Drager BJ, Lotan Y, Jenkins LC, Droller M, van Rhijn BW, Karakiewicz PI, International Consultation on Urologic Disease-European Association of Urology Consultation on Bladder C. ICUD-EAU International Consultation on Bladder Cancer 2012: Screening, diagnosis, and molecular markers. Eur Urol 2013;63:4-15.
3. Esteller M. Epigenetics in cancer. N Engl J Med 2008;358:1148-1159.
4. Kim WJ, Kim YJ. Epigenetic biomarkers in urothelial bladder cancer. Expert review of molecular diagnostics 2009;9:259-269.
5. Besaratinia A, Cockburn M, Tommasi S. Alterations of DNA methylome in human bladder cancer. Epigenetics 2013;8:1013-1022.
6. Kandimalla R, van Tilborg AA, Zwarthoff EC. DNA methylation-based biomarkers in bladder cancer. Nat Rev Urol 2013;10:327-335.
7. Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. Nature Reviews Cancer 2011;11:726-734.
8. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis 2010;31:27-36.
9. Hellebrekers DM, Lentjes MH, van den Bosch SM, Melotte V, Wouters KA, Daenen KL, Smits KM, Akiyama Y, Yuasa Y, Sanduleanu S, Khalid-de Bakker CA, Jonkers D, Weijenberg MP, Louwagie J, van Criekinge W, Carvalho B, Meijer GA, Baylin SB, Herman JG, de Bruine AP, van Engeland M. GATA4 and GATA5 are potential tumor suppressors and biomarkers in colorectal cancer. Clin Cancer Res 2009;15:3990-3997.
10. Peters I, Dubrowinskaja N, Kogosov M, Abbas M, Hennenlotter J, von Klot C, Merseburger AS, Stenzl A, Scherer R, Kuczyk MA, Serth J. Decreased GATA5 mRNA expression associates with CpG island methylation and shortened recurrence-free survival in clear cell renal cell carcinoma. BMC Cancer 2014;14:101-106.
11. Peters I, Eggers H, Atschekzei F, Hennenlotter J, Waalkes S, Trankenschuh W, Grosshennig A, Merseburger AS, Stenzl A, Kuczyk MA, Serth J. GATA5 CpG island methylation in renal cell cancer: a potential biomarker for metastasis and disease progression. BJU Int

2012;110:E144-152.

12. Peters I, Gebauer K, Dubrowinskaja N, Atschekzei F, Kramer MW, Hennenlotter J, Tezval H, Abbas M, Scherer R, Merseburger AS, Stenzl A, Kuczyk MA, Serth J. GATA5 CpG island hypermethylation is an independent predictor for poor clinical outcome in renal cell carcinoma. Oncol Rep 2014;31:1523-1530.
13. Wang D, Yang PN, Chen J, Zhou XY, Liu QJ, Li HJ, Li CL. Promoter hypermethylation may be an important mechanism of the transcriptional inactivation of ARRDC3, GATA5, and ELP3 in invasive ductal breast carcinoma. Mol Cell Biochem 2014;396:67-77.
14. Wang X, Kang GH, Campan M, Weisenberger DJ, Long TI, Cozen W, Bernstein L, Wu AH, Siegmund KD, Shibata D, Laird PW. Epigenetic subgroups of esophageal and gastric adenocarcinoma with differential GATA5 DNA methylation associated with clinical and lifestyle factors. PLoS One 2011;6:e25985.
15. Zhang X, Song YF, Lu HN, Wang DP, Zhang XS, Huang SL, Sun BL, Huang ZG. Combined detection of plasma GATA5 and SFRP2 methylation is a valid noninvasive biomarker for colorectal cancer and adenomas. World J Gastroenterol 2015;21:2629-2637.
16. Agundez M, Grau L, Palou J, Algaba F, Villavicencio H, Sanchez-Carbayo M. Evaluation of the methylation status of tumour suppressor genes for predicting bacillus Calmette-Guerin response in patients with T1G3 high-risk bladder tumours. Eur Urol 2011;60:131-140.
17. Sacristan R, Gonzalez C, Fernandez-Gomez JM, Fresno F, Escaf S, Sanchez-Carbayo M. Molecular classification of non-muscle-invasive bladder cancer (pTa low-grade, pT1 low-grade, and pT1 high-grade subgroups) using methylation of tumor-suppressor genes. J Mol Diagn 2014;16:564-572.
18. Babjuk M, Oosterlinck W, Sylvester R, Kaasinen E, Bohle A, Palou-Redorta J. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder. Eur Urol 2008;54:303-314.
19. Merika M, Orkin SH. DNA-binding specificity of GATA family transcription factors. Mol Cell Biol 1993;13:3999-4010.
20. Patient RK, McGhee JD. The GATA family (vertebrates and invertebrates). Curr Opin Genet Dev 2002;12:416-422.
21. Graf T, Enver T. Forcing cells to change lineages. Nature 2009;462:587-594.
22. Gao X, Sedgwick T, Shi YB, Evans T. Distinct functions are implicated for the GATA-4, -5, and -6 transcription factors in the regulation of intestine epithelial cell differentiation. Mol Cell Biol 1998;18:2901-2911.
23. Fujikura J, Yamato E, Yonemura S, Hosoda K, Masui S, Nakao K, Miyazaki Ji J, Niwa H. Differentiation of embryonic stem cells is induced by GATA factors. Genes Dev 2002;16:784-789.
24. Zhou W, Goodman SN, Galizia G, Lieto E, Ferraraccio F, Pignatelli C, Purdie CA, Piris J, Morris R, Harrison DJ, Paty PB, Culliford A, Romans KE, Montgomery EA, Choti MA, Kinzler KW, Vogelstein B. Counting alleles to predict recurrence of early-stage colorectal cancers. Lancet 2002;359:219-225.