The role of histone H3K27 dimethylation in early-stage urinary bladder carcinoma: the relevance of risk factor

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Background
Epigenetic alterations, including post-translational modification of histone tails by methylation may play an important role in carcinogenesis.
Objective
The aim was to evaluate the global histone H3K27 dimethylation (H3K27me2) levels in bladder cancer (BC) and to compare these levels in different types and stages of BC.
Materials and Methods
Venous blood from 45 BC patients and 45 apparently healthy controls was used. The two risk factors such as *Schistosoma haematobium* infection and smoking were investigated. Histone extraction was done and used to determine the global levels of H3K27 dimethylation.
Results
Global level of H3K27 dimethylation was significantly lower in BC patients than in healthy controls. We observed a negative correlation between histone dimethylation levels and the smoking state (both in patients and controls). Receiver operating characteristic curve showed that histone H3K27me2 at a cut-off point less than 49.68 ng/μl has 69% sensitivity and 64.5% specificity for the prediction of BC with an area under the curve of 0.67 ($P = 0.001$). However, there was no statistically significant difference in H3K27me2 levels as regards history of *S. haematobium* infection ($P = 0.6$), histopathological types ($P = 0.3$), and the stages of cancer ($P = 0.8$).
Conclusion
The global histone H3K27 dimethylation may substantiate the potential to improve the detection of early-stage urinary bladder carcinoma. Also, the decreased level of histone H3K27me2 in smokers (either patients or controls) could be one entity that explain smoking as a risk factor for BC.

Keywords:
bladder carcinoma, histone extraction, histone methylation, *Schistosoma haematobium*, smoking

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Introduction

Bladder cancer (BC) is the seventh most common cancer worldwide and the second most common cause of death in patients suffering from genitourinary tract malignancies [1,2]. The relative frequency of BC in Egypt is 10.7% among men and 3% among women at the National Cancer Registry of Egypt in 2014 [3]. Urothelial carcinomas, arising from superficial cell layers in the urogenital tract, account for more than 90% of BC cases with the remainder of squamous cell carcinomas (SCC) and adenocarcinomas [4].

Tobacco smoking and occupational exposure to aromatic amines are the main environmental risk factors for BC occurrence in Western countries [5]. However, *Schistosoma haematobium* infection is the most accused in developing countries, particularly in Egypt [6].

Histones are basic protein molecules of the chromatin, where coiled DNA is wrapped around. Each of the histones has a tail extension that can be modified by a number of histone post-translational modifying enzymes resulting in methylation, acetylation, phosphorylation, and ubiquitination [7]. These modifications usually alter the binding affinity of the tails to DNA, resulting in the alteration of gene transcription, DNA replication, DNA repair, in addition to organization of chromosomes [8].

Histone methylation is the addition of one, two, or three methyl groups mostly at lysines (K) and arginines (R) amino acids [9]. Lysines could be monomethylated, dimethylated, or trimethylated, while arginines are only monomethylated or dimethylated by histone-methyltransferases and histone-demethylases [9,10].

Methylation of lysine residue 27 of histone H3 (H3K27) may induce the heterochromatin formation by recruiting polycomb group (PcG) proteins. On the other hand, demethylation of trimethylated histone H3K27 (H3K27me3) depressed and promoted gene expression to change heterochromatin into euchromatin [11]. Consequently, H3K27 is one of the histone methylation targets that are implicated in transcription repression of neighboring genes [12].

Several studies have indicated that global levels of histone modifications are suitable cancer biomarkers [13–15]. However, H3K27 methylation levels were inadequately investigated in BC patients. The aim of this study was to determine the global level of histone H3K27 dimethylation (H3K27me2) as a potential biomarker for early detection of BC.

Materials and methods

Patients

A total of 90 participants were prospectively recruited and classified into two groups. Group I included 45 BC patients, who attended the Surgical Oncology Department, South Egypt Cancer Institute from November 2016 to October 2017. Only BC patients with stage 1 and 2 tumors according to the TNM (primary tumor/lymph nodes/distant metastasis) staging system for BC were included [16]. Smoking state and history of schistosomal infections were also investigated as risk factors for BC. Patients with advanced BC (stages 3 and 4), history of other cancers, acute or chronic liver or renal disease, connective tissue disease, and metabolic disease were excluded. Group II included 45 apparently healthy, age-matched volunteers. They were not receiving regular medications and had no evidence of neoplastic or chronic inflammatory disease after a careful history taking and clinical examination. Smokers included in our study are defined as heavy smokers, who are consuming greater than or equal to 1 pack daily (20 Egyptian cigarettes).

The study protocol was approved by the Ethics Committee of Assiut University and according to The Code of Ethics of the World Medical Association (Declaration of Helsinki). All the participants gave written informed consents before their participation.

The patients were subjected to complete medical history taking, physical examination, MRI, computed tomography, cystoscopy, and pathological examination of the excised tumor. The histopathological studies were done at the Cancer Pathology Department, South Egypt Cancer Institute. The practical part was done in the Medical Research Centre, Faculty of Medicine, Assiut University, Egypt.

Methods

Sample collection

A volume of 2 ml of venous blood was collected from both patients and controls into EDTA tubes and stored in −80°C until the histone extraction procedure. Blood samples for routine investigation were also collected.

Total histone extraction from the samples

Total histone extraction was done using the EpiQuik Total Histone Extraction Kit (Catalog # OP-0006, made in USA). Histone extraction was done according to the kit protocol, with some modifications where whole blood was used instead of isolated blood cells as a new method. Briefly, blood samples were treated by an equal volume of pre-lysis buffer, centrifuged, and the supernatant were removed. Pellets were resuspended in lysis buffer, incubated and then centrifuged where the supernatant fraction (containing acid-soluble proteins) was transferred into a new vial. Lastly, balance-DTT buffer was added to the supernatant immediately. The protein concentration was measured with an OD reading. Bovine serum albumin was used as a standard. The extracted histone was used for the detection of global histone H3K27 dimethylation level.

Determination of the global histone H3K27 dimethylation levels

Global histone H3K27 dimethylation levels were determined using the EpiQuik Global Di-Methyl Histone H3K27 Quantification Colorimetric Kit (Catalog # P-3040, made in USA). Briefly, the dimethylated histone H3 at lysine 27 is captured to wells coated with an antidimethyl H3K27 antibody. Then, H3K27 dimethylated histone can be detected with a labeled antibody, followed by a color development reagent.

Statistical analysis of data

Sample size calculation was conducted using the G Power (University of Kiel, Kiel, Germany) program in order to detect significant difference in the proportion of histone H3K27 dimethylation levels between two independent groups included in this study with power 0.95 in a hypothetical effect size of 0.33. Each group had 45 samples with a total sample size of 90. Data were collected and analyzed using SPSS (statistical package for the social sciences, version 20; IBM, Armonk, New York, USA). Continuous data were

expressed as mean ± SD and range while nominal data were expressed in the form of frequency (percentage). χ^2-Test was used to compare the nominal data of both studied groups while Student's *t*-test or one-way analysis of variance test was used to compare the means of continuous variables between both study and control groups. A *P* value) of less than 0.05 was considered statistically significant. The threshold value for optimal sensitivity and specificity of the histone H3K27 dimethylation level were determined by the receiver operating characteristic (ROC) curve.

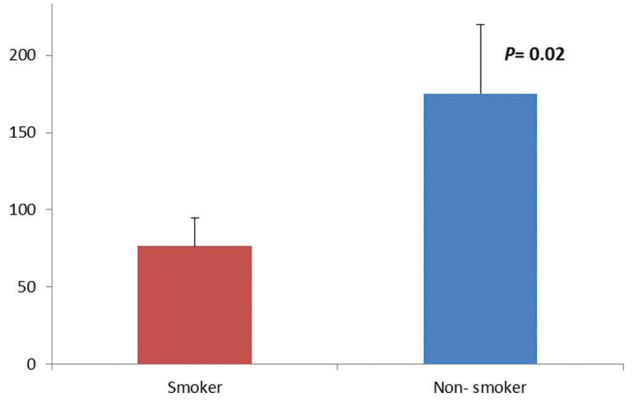
Results

Demographic data of the studied groups is shown in Table 1. The main presenting symptom in the studied BC patients was hematuria that exist in 37 (82.2%) patients while hematuria and dysuria were presented only in eight (17.8%) patients. Disease characteristics of the patients are shown in Table 2. Most of the patients had high grade carcinogenesis, 43 (95.6%) patients and only two (4.4%) patients had low grade of malignancy.

Patients with BC had significantly lower levels of histone H3K27me2 in comparison to the control group as shown in Table 3. However, there was no statistically significant difference in H3K27me2 levels as regards the grades (P = 0.9) and histopathological types (P = 0.3) and the stage of cancer (P = 0.8), as shown in Table 2. It was noticed that smokers, whether BC patients or apparently healthy individuals, had lower levels of histone H3K27me2 in comparison to those who were nonsmokers in all subjects with significant difference (76.55 ± 17.88 vs. 175.03 ± 57.09; P = 0.02) as shown in Fig. 1. In addition, there was no significant difference in the histone H3K27me2 level in smoker patients as regards histological types (P = 0.7). BC patients with a history of bilharziasis had a higher level of histone H3K27me2 in comparison to those without history of bilharziasis, but the *P* value was insignificant (56.9 ± 10.7 vs. 53.1 ± 13.1; P = 0.6) as shown in Table 2.

The ROC curve showed that histone H3K27me2 level at a cut-off point of less than 49.68 ng/µl had 69% sensitivity and 65% specificity for the prediction of BC with the area under the curve = 0.67 (P = 0.001) as shown in Fig. 2.

Figure 1



Level of histone H3K27me2 in all participants based on smoking state. Smokers, whether bladder cancer patients or apparently healthy control, had lower levels of histone H3K27 dimethylation in comparison to those who were nonsmokers with significant difference (P = 0.02). H3K27me2: histone H3K27 dimethylation.

Table 1 Demographic data of the studied groups

	Bladder cancer group (*n*=45)	Control group (*n*=45)	*P*
Age (years)			
Mean±SD	57.27±9.14	49.38±7.56	0.09
Range	39-75	30-65	
Sex			
Male	30 (66.7)	37 (82.2)	0.1
Female	15 (33.3)	8 (17.8)	
Marital status			
Single	2 (4.4)	7 (15.6)	0.3
Married	43 (95.6)	38 (84.4)	
Smoking	24 (53.3)	20 (44.4)	0.7
Schistosmal infection	15 (33.3)	0	0.00
Comorbidities			
Hypertension	21 (46.7)	17 (37.7)	0.7
Diabetes mellitus	15 (33.3)	14 (31.11)	0.9
Laboratory data of the studied groups			
Hemoglobin (g %)	11.93±1.63	12.86±0.91	0.001
Random blood sugar (mg/dl)	147.75±32.02	123.01±12.02	0.98
Blood urea nitrogen (mg/dl)	31.09±9.57	14.82±4.14	0.001
Serum creatinine (mg/dl)	1.22±0.50	0.85±0.19	0.001

Continuous data were expressed in the form of mean±SD (compared with Student's *t*-test) while nominal data were expressed in the form of frequency (percentage) (compared with the χ^2-test). *P* value was significant if <0.05.

Table 2 Disease characteristics and histone H3K27me2 level in bladder cancer patients

	n=45	Level of histone H3K27me2 (ng/μl)	
		Mean±SD	*P*
Stage			
T1	19 (42.2)	55.6±15.1	0.8
T2	26 (57.8)	54.3±8.9	
Grade			
High	43 (95.6)	54.78±25.67	0.9
Low	2 (4.4)	53.78±2.89	
Histopathology			
Squamous cell carcinoma	11 (24.4)	55.5±8.1	0.3
Urothelial carcinoma	28 (62.2)	52.6±9.5	
Urothelial carcinoma with squamous differentiation	6 (13.3)	54.1±9.9	
Smoking state			
Smokers	24 (53.3)	49.8±11.8	0.04
Nonsmokers	21 (46.7)	65.9±16. 1	
History of *Schistosoma haematobium* infection			
Positive	15 (33.3)	56.9±10.7	0.6
Negative	30 (66.7)	53.1±13. 1	

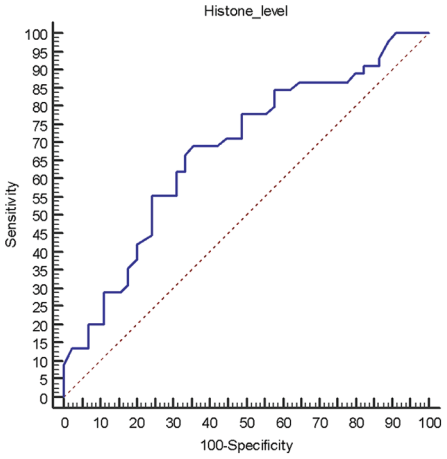
Continuous data were expressed in the form of mean±SD (compared with Student's *t*-test) while nominal data were expressed in the form of frequency (percentage) (compared with the χ^2-test). BC, bladder cancer; H3K27me2, histone H3K27 dimethylation; T1, stage 1 bladder cancer; T2, stage 2 bladder cancer estimated according to the TNM (primary tumor/lymph nodes/distant metastasis) staging system for bladder cancer (16). *P* value was significant if <0.05.

Table 3 Level of histone H3K27 dimethylation in bladder cancer patients and controls as whole, and based on the smoking state

Histone H3K27me2 (ng/μl)	Bladder cancer (*n*=45)	Control group (*n*=45)	*P*
Mean±SD	54.87±12.3	156.78±35.09	P_1=0.001
Smokers	*n*=24	*n*=20	
Mean±SD	49.83±11.77	141.08±25.11	P_2=0.001
Nonsmokers	*n*=21	*n*=25	
Mean±SD	65.91±16.01	166.43±25.45	P_3=0.001

Continuous data were expressed in the form of mean±SD (compared with Student's *t*-test). H3K27me2, histone H3K27 dimethylation; P_1, difference in H3K27 dimethylation levels between the diseased and control groups; P_2, difference in H3K27 dimethylation levels between smokers in each group; P_3, difference in H3K27 dimethylation levels between nonsmokers in each group. *P* value was significant if <0.05.

Figure 2



ROC curve for the prediction of bladder cancer based on histone H3K27me2 level. The ROC curve showed that histone H3K27 dimethylation at a cut-off point of less than 49.68 ng/μl has 69% sensitivity and 64.44% specificity for the prediction of bladder cancer with an area under the curve of 0.67 (*P* = 0.001). ROC, receiver operating characteristic; AUR: area under the curve.

Discussion

BC represents one of the most common urological carcinomas around the world and is the seventh most frequent cancer in the world [2]. Classical urine cytology and cystoscopy are mainly used for the surveillance of patients with BC. However, the decreased sensitivity of voided urine cytology for low-grade tumor, the invasiveness of cystoscopy [17,18], together with the frequent recurrences rate of BC, this necessitates the development of a sensitive noninvasive diagnostic test that could specifically detect BC in the early stages [17–19].

Alteration in the cancer cells global histone modification level and its prognostic efficacy was demonstrated for numerous human malignancies [20]. The H3K27 methylation allowed differentiation of the malignant tissue from normal one in various urological malignancies such as prostate cancer [21], penile carcinoma [11], and renal cell carcinoma [22]. However, H3K27 methylation levels have not been widely investigated in BC patients.

In the current study, we clarified that the H3K27 dimethylation level was significantly decreased (*P* = 0.001) in BC patients compared with the healthy control. However, there was no statistically significant difference in H3K27me2 levels as regards the grades (*P* = 0.9) histopathological types (*P* = 0.3), and the stages (first and second stage) of cancer (*P* = 0.8). These findings are in accordance with other studies that illustrated a decrease in histone

methylation levels of many cancers. Ellinger *et al.* [15] documented that global levels of H3K9 and H3K27 methylation were significantly higher in healthy control than in BC, and in nonmuscle invasive BC compared with muscle-invasive BC. Moreover, Rogenhofer *et al.* [22] found an inverse correlation of H3K27me1, H3K27me2, H3K27me3 levels with Fuhrman grading and pathological T-stages in renal cell carcinomas.

Similarly, global levels of H3K4me1, H3K9me1, H3K9me2, H3K27me2, and H3K27me3 were reported to be decreased in penile SCC [11]. Additionally, the level of trimethylated H3K27 was lower in renal cell cancer tissues compared with normal tissues [23]. Pellakuru *et al.* [21] established that global levels of H3K27me3 were decreased in prostatic intraepithelial neoplasia and invasive adenocarcinoma lesions that correlate with increased markers of disease aggressiveness (e.g. Gleason score and pathological stage). Also, global levels of H3K4 and H4K20 methylation were decreased in BC compared with normal urothelium tissue, in metastatic tumor than in the primary tumor [24]. As well, Ellinger *et al.* [25] reported that H3K4me1, H3K9me2, and H3K9me3 were significantly reduced in prostate carcinoma.

Although, histone methylation does not amend the charge of the histone protein, the addition of methyl groups to histone residues creates steric bulkiness and removes a potential hydrogen bond donor, thus disturbing the interactions between DNA and histones and consequently disrupt the chromatin structure [26,27]. This may result in alteration of gene transcription, DNA repair, and DNA replication, in addition to the organization of chromosomes [8,28,29]. Moreover, methylation of histones is considered to be a critical step involved in many cell fate determinations, including cell differentiation and development processes, pluripotency, and maintenance of genome integrity [30–32]. However, the site of histone methylation is causative for its effect on transcriptional activity, as transcriptional activation is characterized by methylation at H3K4, H3K36 or H3K79, while methylation at H3K9, H3K27, and H4K20 is associated with transcriptional repression [33]. H3K27 is known for one action: shutting down transcription as it is tightly associated with and inactivates gene promoters [12].

In addition to its diagnostic role, several studies have shown that global levels of histone modifications are of predictive value for the outcome, as the lower the histone methylation levels, the worse the prognosis and the outcome. H3K27me2 levels were found to be considerably lower in liver metastases than in the corresponding primary colorectal cancer [34], while increased H3K27me3 levels were associated with better prognosis and survival in numerous breast tumor subtypes [35]. Furthermore, Rogenhofer *et al.* [11] recognized that decreased H3K9me1 levels indicate poor prognosis in patients with renal cell carcinoma. Moreover, Ellinger *et al.* [36] reported that H3K27me1 is also of prognostic value as its levels were correlated with pathological T-stage, capsular infiltration, seminal vesicle penetration, and Gleason score in localized prostate carcinomas.

Contrary of our results, some studies reported an association between elevation of histone methylation levels and bad prognosis in different cancers, while He *et al.* [37] correlated the high expression of H3K27me3 in esophageal SCC with poor prognosis. Tzao *et al.* [38] correlated it with nodal status and stage of the carcinoma. Besides, high expression of H3K27me3 in hepatocellular carcinoma was positively correlated with large tumor size, multiplicity, poor differentiation, advanced stage, vascular invasion, and short survival [39]. Moreover, high level of H3K9me3 in gastric adenocarcinoma was associated with higher T stage, lymphovascular invasion, high recurrence rate, and a poor survival rate [40]. Finally, Benard *et al.* [41] explored a high expression of H3K4me3 in early-stage colon cancer that correlated with shorter patient survival and higher recurrence rate.

Additionally, we demonstrated a significant difference in H3K27 dimethylation levels concerning smoking state, as smokers (both patients and controls) had lower levels of histone H3K27 dimethylation in comparison to those who were nonsmokers ($P = 0.02$). To the best of our knowledge, we recognize our self to be the first that studied the effect of cigarette smoking in modulating histone H3K27me2 levels in BC. However, our results are in accordance with others who hypothesized that cigarette smoking exposure in mouse and human bronchial epithelial cells (H292) may induce a site-specific post-translational histone modifications (PTMs), which may be implicated in the pathogenesis of cigarette smoking-induced chronic lung diseases [42]. In addition, Ibuki *et al.* [43] reported that cigarette sidestream smoke created the phosphorylation of H3S10, where the promoter region of proto-oncogenes existed, leading to tumor promotion. These signals were mainly arbitrated via the JNK pathways and partly through the PI3K/Akt pathway [43].

In conclusion, this study demonstrated that histone H3K27me2 level was decreased in BC patients compared with apparently healthy control; therefore, assessment of histone H3K27 dimethylation levels could be clinically helpful in early diagnosis of bladder

carcinomas using noninvasive biomarkers from blood samples. In addition, the assessment of histone H3K27me2 levels could be used to exclude advanced stage BC if the histone H3K27 dimethylation level is above 50 ng/μl. Besides, it may be considered as a noninvasive blood-based marker for the follow-up of BC patients after cystectomy as it could exclude recurrence as well as the histone H3K27me2 level of more than 50 ng/μl. Furthermore, the decrease in the histone H3K27me2 level in smoker patients and in all smokers (both patients and controls) could be one of the possible mechanisms that explain smoking as risk factor for BC.

Recommendation

The authors recommend that further studies with larger numbers of BC patients and follow-up are needed to explain the prognostic role of histone H3K27 dimethylation in BC. Also, additional studies are desirable to clarify the relationship between smoking and epigenetic aberration in BC.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Eissa S, Shawky SM, Matboli M, Mohamed S, Azzazy HM. Direct detection of unamplified hepatoma upregulated protein RNA in urine using gold nanoparticles for bladder cancer diagnosis. Clin Biochem 2014; 47:104–110.

2 Ye T, Ding W, Wang N, Huang H, Pan Y, Wei A. Long noncoding RNA linc00346 promotes the malignant phenotypes of bladder cancer. Biochem Biophys Res Commun 2017; 491:79–84.

3 Ibrahim AS, Khaled HM, Mikhail NN, Baraka H, Kamel H. Cancer incidence in Egypt: results of the national population-based cancer registry program. J Cancer Epidemiol 2014; 2014:18.

4 Dinney CP, Fisher MB, Navai N, O'donnell MA, Cutler D, Abraham A, *et al*. Phase I trial of intravesical recombinant adenovirus mediated interferon-α2b formulated in Syn3 for Bacillus Calmette-Guérin failures in nonmuscle invasive bladder cancer. J Urol 2013; 190:850–856.

5 Cumberbatch MG, Rota M, Catto JW, La Vecchia C. The role of tobacco smoke in bladder and kidney carcinogenesis: a comparison of exposures and meta-analysis of incidence and mortality risks. Eur Urol 2016; 70:458–466.

6 Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder cancer incidence and mortality: a global overview and recent trends. Eur Urol 2017; 71:96–108.

7 Qin W, Wolf P, Liu N, Link S, Smets M, La Mastra F, *et al*. DNA methylation requires a DNMT1 ubiquitin interacting motif (UIM) and histone ubiquitination. Cell Res 2015; 25:911.

8 Kumar P, Periyasamy R, Das S, Neerukonda S, Mani I, Pandey KN. All-trans retinoic acid and sodium butyrate enhance natriuretic peptide receptor a gene transcription: role of histone modification. Mol Pharmacol 2014; 85:946–957.

9 Ahmed AA, Musa HH, Sifaldin AZ, Musa TH. Epigenetic events in male common urogenital organs cancer. J Cancer Res Pract 2016; 3:104–112.

10 Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, *et al*. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nat Genet 2010; 42:722.

11 Rogenhofer S, Miersch H, Göke F, Kahl P, Wieland WF, Hofstädter F, *et al*. Histone methylation defines an epigenetic entity in penile squamous cell carcinoma. J Urol 2013; 189:1117–1122.

12 Barski A, Cuddapah S, Cui K, Roh T-Y, Schones DE, Wang Z, *et al*. High-resolution profiling of histone methylations in the human genome. Cell 2007; 129:823–837.

13 Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. Nat Rev Genet 2012; 13:343.

14 Chervona Y, Costa M. Histone modifications and cancer: biomarkers of prognosis? Am J Cancer Res 2012; 2:589.

15 Ellinger J, Bachmann A, Göke F, Behbahani TE, Baumann C, Heukamp LC, *et al*. Alterations of global histone H3K9 and H3K27 methylation levels in bladder cancer. Urol Int 2014; 93:113–118.

16 Cheng L, Montironi R, Davidson DD, Lopez-Beltran A. Staging and reporting of urothelial carcinoma of the urinary bladder. Mod Pathol 2009; 22:S70–S95.

17 Eissa S, Shabayek MI, Ismail MF, El-Allawy RM, Hamdy MA. Diagnostic evaluation of apoptosis inhibitory gene and tissue inhibitor matrix metalloproteinase-2 in patients with bladder cancer. IUBMB Life 2010; 62:394–399.

18 Eissa S, Swellam M, Amin A, Balbaa ME, Yacout GA, El-Zayat TM. The clinical relevance of urine-based markers for diagnosis of bladder cancer. Med Oncol 2011; 28:513–518.

19 Hutterer GC, Karakiewicz PI, Zippe C, Lüdecke G, Boman H, Sanchez-Carbayo M, *et al*. Urinary cytology and nuclear matrix protein 22 in the detection of bladder cancer recurrence other than transitional cell carcinoma. BJU Int 2008; 101:561–565.

20 Kurdistani SK. Histone modifications in cancer biology and prognosis. Prog Drug Res 2011; 67:91–106.

21 Pellakuru LG, Iwata T, Gurel B, Schultz D, Hicks J, Bethel C, *et al*. Global levels of H3K27me3 track with differentiation *in vivo* and are deregulated by MYC in prostate cancer. Am J Pathol 2012; 181:560–569.

22 Rogenhofer S, Kahl P, Mertens C, Hauser S, Hartmann W, Büttner R, *et al*. Global histone H3 lysine 27 (H3K27) methylation levels and their prognostic relevance in renal cell carcinoma. BJU Int 2012; 109:459–465.

23 Shen Y, Guo X, Wang Y, Qiu W, Chang Y, Zhang A, *et al*. Expression and significance of histone H3K27 demethylases in renal cell carcinoma. BMC Cancer 2012; 12:470.

24 Schneider AC, Heukamp LC, Rogenhofer S, Fechner G, Bastian PJ, von Ruecker A, *et al*. Global histone H4K20 trimethylation predicts cancer-specific survival in patients with muscle-invasive bladder cancer. BJU Int 2011; 108:8b.

25 Ellinger J, Kahl P, von der Gathen J, Rogenhofer S, Heukamp LC, Gütgemann I, *et al*. Global levels of histone modifications predict prostate cancer recurrence. Prostate 2010; 70:61–69.

26 Bannister A, Kouzarides T. Regulation of chromatin by histone modifications. Cell Res 2011; 21:381–395.

27 Srivastava R, Ahn SH. Modifications of RNA polymerase II CTD: Connections to the histone code and cellular function. Biotechnol Adv 2015; 33:856–872.

28 Hunt CR, Ramnarain D, Horikoshi N, Iyengar P, Pandita RK, Shay JW, *et al*. Histone modifications and DNA double-strand break repair after exposure to ionizing radiations. Radiat Res 2013; 179:383–392.

29 Abmayr SM, Workman JL. Holding on through DNA replication: histone modification or modifier? Cell 2012; 150:875–877.

30 Pasini D, Bracken AP, Hansen JB, Capillo M, Helin K. The polycomb group protein Suz12 is required for embryonic stem cell differentiation. Mol Cell Biol 2007; 27:3769–3779.

31 Torres-Padilla M-E, Parfitt D-E, Kouzarides T, Zernicka-Goetz M. Histone arginine methylation regulates pluripotency in the early mouse embryo. Nature 2007; 445:214.

32 Schotta G, Sengupta R, Kubicek S, Malin S, Kauer M, Callén E, *et al*. A chromatin-wide transition to H4K20 monomethylation impairs genome integrity and programmed DNA rearrangements in the mouse. Genes Dev 2008; 22:2048–2061.

33 Kouzarides T. Chromatin modifications and their function. Cell 2007; 128:693–705.

34 Tamagawa H, Oshima T, Numata M, Yamamoto N, Shiozawa M, Morinaga S, *et al*. Global histone modification of H3K27 correlates with the outcomes in patients with metachronous liver metastasis of colorectal cancer. Eur J Surg Oncol 2013; 39:655–661.

35 Holm K, Grabau D, Lövgren K, Aradottir S, Gruvberger-Saal S, Howlin J, *et al*. Global H3K27 trimethylation and EZH2 abundance in breast tumor subtypes. Mol Oncol 2012; 6:494–506.

36 Ellinger J, Kahl P, von der Gathen J, Heukamp LC, Gütgemann I, Walter B, *et al*. Global histone H3K27 methylation levels are different in localized and metastatic prostate cancer. Cancer Invest 2012; 30:92–97.

37 He L-R, Liu M-Z, Li B-K, Rao H-L, Liao Y-J, Guan X-Y, *et al*. Prognostic impact of H3K27me3 expression on locoregional progression after chemoradiotherapy in esophageal squamous cell carcinoma. BMC Cancer 2009; 9:461.

38 Tzao C, Tung H-J, Jin J-S, Sun G-H, Hsu H-S, Chen B-H, *et al*. Prognostic significance of global histone modifications in resected squamous cell carcinoma of the esophagus. Mod Pathol 2009; 22:252.

39 Cai M-Y, Hou J-H, Rao H-L, Luo R-Z, Li M, Pei X-Q, *et al*. High expression of H3K27me3 in human hepatocellular carcinomas correlates closely with vascular invasion and predicts worse prognosis in patients. Mol Med 2011; 17:12.

40 Park YS, Jin MY, Kim YJ, Yook JH, Kim BS, Jang SJ. The global histone modification pattern correlates with cancer recurrence and overall survival in gastric adenocarcinoma. Ann Surg Oncol 2008; 15:1968.

41 Benard A, Goossens-Beumer IJ, van Hoesel AQ, de Graaf W, Horati H, Putter H, *et al*. Histone trimethylation at H3K4, H3K9 and H4K20 correlates with patient survival and tumor recurrence in early-stage colon cancer. BMC Cancer 2014; 14:531.

42 Sundar IK, Nevid MZ, Friedman AE, Rahman I. Cigarette smoke induces distinct histone modifications in lung cells: implications for the pathogenesis of COPD and lung cancer. J Proteome Res 2013; 13:982–996.

43 Ibuki Y, Toyooka T, Zhao X, Yoshida I. Cigarette sidestream smoke induces histone H3 phosphorylation via JNK and PI3K/Akt pathways, leading to the expression of proto-oncogenes. Carcinogenesis 2014; 35:1228–1237.