

<http://ansinet.com/itj>

ITJ

ISSN 1812-5638

INFORMATION TECHNOLOGY JOURNAL

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

DNA Methylation-based Biomarkers in Urological Cancers

^{1,2}Rong Xu, ³Shaokun Lin, ³Jun Xin, ²Youhong Guo, ²Wenzhou Zhang, ²Yangjun Lin and ¹Rui'an Xu

¹Institute of Molecular Medicine, Huaqiao University, Quanzhou 362021, China

²Department of Pharmacy, Quanzhou Medical College, Quanzhou 362011, China

³Department of Urology, the First Hospital of Quanzhou Affiliated Fujian Medical University, Quanzhou, Fujian 362000, China

Abstract: The formation of the urological cancers is a complicated process including a large number of gene modifications. There are two key mechanisms in this process, genetics and epigenetics. Genetics of the Urinary system cancer mutation formation by means of DNA nucleotide sequences alteration; Epigenetics of urological cancers the levels of genes change through base modification without DNA nucleotide sequences alteration. As the major member of epigenetic family, DNA methylation changes the normal expression of the genes, playing an important role in the urological cancers genesis and development. DNA methylation including the hypermethylation of tumor suppressor genes and the hypomethylation of some tumor genes. We reviewed recent advances in the research on DNA methylation in the urological cancers (Renal cell carcinoma, Bladder cancer and Prostate cancer), summarized the methylation profile of the urological cancers related genes, including the hypermethylation of tumor suppressor genes and the hypomethylation of some tumor genes. Intensive study on DNA methylation would provide a new opportunity for the early diagnosis, prognosis and treatment of the urological cancers.

Key words: Epigenetics, DNA Methylation, hypermethylation, hypomethylation, renal cell carcinoma, bladder cancer, prostate cancer

INTRODUCTION

Recent studies have found that almost all human tumors are made with genetically altered epigenetic abnormalities common cause and promote its evolution. Epigenetic concept is proposed in 1942 by Waddington. Epigenetic modifications that affect gene transcription activity thereby affecting the phenotype, but do not involve changes in the DNA sequence of the gene expression and regulation, including changes in the DNA methylation status, histone modifications and chromatin remodeling and small interfering RNA molecules, which DNA methylation is currently the most studied, most clearly epigenetic manner. Epigenetic modifications in tumor incidence, diagnosis and treatment are important. Cause abnormal epigenetic gene expression by mistake. Cause metabolic disorders and diseases and even cancer. Studies on DNA methylation is conducive to further clarify urinary tumor formation and the development of epigenetic mechanisms. Therefore, as the urological cancers "second strike" an important complement to the classic hypothesis, DNA methylation research in recent years has become one of the hot (Jones and Laird, 1999).

Urological cancers are occurred in any part of the urological cancers, including kidney, renal pelvis, ureter,

Table 1: Biomarkers in urological cancers

Tumor	Kidney cancer	Bladder cancer	Prostate cancer
Hypermethylation	GSTP1	XPC	
	MGMT	GSTP1	GSTP1
	P16	RASSF1A	CDH1
	RASSF1A	CDH1	APC
	TIMP3	DAPK	AR
	APC	p16	RASS1A
	CDH1	BCL2	cyclin D2
	DAPK	hTERT	
Hypomethylation	EDNRB		
	HPSE	WNT5A	CRIP1
	CA9	LINE1	S100P
			LINE-1

bladder, urethra cancer and prostate cancer. Bladder cancer is the most common, followed by kidney tumors, Europe and the United States the most common prostate cancer is relatively rare in our country, but there are obvious growth trend. DNA hypermethylation in tumorigenesis and urinary system also plays an important role in the development, DNA methylation abnormalities can affect chromatin structure and oncogenes and tumor suppressor genes involved in tumor formation (Zhu and Yao, 2009). Thus, DNA methylation and urological cancers research in recent years become a hot spot. The DNA Methylation-based Biomarkers in Urological Cancers are shown in Table 1.

KIDNEY CANCER GENE METHYLATION

Renal cell carcinoma is the most common urological malignancy of the urological cancers incidence rate in the second place, accounting for systemic cancer 2 to 3%. Kidney cancer and the formation of a multi-Genetically modified complex process in which both genetic and epigenetic mechanisms play an important role. While the latter to DNA methylation most detailed study. Many studies have confirmed the occurrence of kidney cancer, development and aberrant DNA methylation-related.

Kidney cancer gene hypermethylation: Numerous studies show that the formation and occurrence of kidney cancer and abnormal DNA methylation has a close relationship, kidney cancer, including DNA hypermethylation gene promoter region methylation and certain oncogenes hypomethylation. The former refers to the unmethylated gene promoter CpG islands are methylated causing many inactivation of tumor suppressor gene silencing; latter refers not normally expressed gene methylation demethylation. Both of these two can make a normal genome methylation status is destroyed, then there abnormal gene expression, leading to uncontrolled cell proliferation, promote tumor development and progression (Jones and Baylin, 2002). RCC common prone DNA hypermethylation of genes mainly include the following: (1) DNA repair gene: Essel Dulaimi (Ellinger *et al.*, 2011) and other cases of renal cell carcinoma in 100 patients found that 12% of patients with GSTP1 (glutathione sulfur transferase P1) the presence of high methylation; Laing *et al.* (2010) found that cancer patients MGMT (6-methylguanine DNA methyltransferase) and P16 (cyclin-dependent kinase inhibitor CDKIs family members) were the presence of high methylation, while the control group was not found methylated. (2) Tumor suppressor gene, De Martino *et al.* (2012) found that 45.9% of renal cell carcinoma patients and 7% of benign tumor suppressor gene RASSF1A (Ras association domain family 1 gene) exist hypermethylated RASSF1A and that is expected to become the diagnosis of renal cell cancer biomarkers; correlation detected papillary renal cell carcinoma TIMP3 (human matrix metalloproteinase inhibitor), APC (adenomatous colon polyps cancer inhibitor) showed high methylation status (Ellinger *et al.*, 2011). (3) cell adhesion and migration of genes, the researchers found that papillary renal cell carcinoma CDH1 (E-cadherin, cadherin E) showed high methylation status, also found closely associated with pathological stage (Ellinger *et al.*, 2011). (4) cell cycle and apoptosis-related genes, Morris *et al.* (2003) found that renal cell carcinoma

DAPK (death-associated protein kinase) showed hypermethylation. (5) signal transduction genes, Pflug *et al.* (2007) found that endothelin receptor gene EDNRB (endothelin B receptor gene) exist hypermethylation (RCC 32/48, renal cancer cell lines 4/6), while in surgery and autopsies were not found in normal kidney tissue EDNRB methylation.

Kidney cancer gene hypomethylation: Genomic DNA hypomethylation and genomic instability associated with an increased rate, CpG Island nucleotide hypomethylation is a human tumor epigenetic changes. Recent studies show that genomic DNA hypomethylation associated with the development of renal cell carcinoma. Garnick (1997) found that renal cell carcinoma IL8 (interleukin-8) hypomethylation high degree than normal tissue, the study also found that the level of DNA hypomethylation and clinical pathological state no correlation. Another study found that CA9 (carbonic anhydrase 9) hypomethylation may be involved in the process of renal cell carcinoma (Cho *et al.*, 2001).

BLADDER CANCER GENE METHYLATION

Bladder cancer is the most common malignancy urinary system, is one cause of death in patients with urinary tract cancer, the second largest tumors (Jemal *et al.*, 2011). Bladder cancer incidence is generally considered to be a polygenic, multifactorial, multi-step synergy. Many studies have confirmed abnormal DNA methylation and bladder cancer occurrence and development.

Bladder cancer gene hypermethylation: Current research indicates that many of bladder cancer were unusually high prevalence of DNA methylation status, this type of gene hypermethylation was no significant difference, the study also found that levels of DNA methylation may be associated with malignant tumors (Dudziec *et al.*, 2011). Common bladder cancer -prone DNA hypermethylation of genes include the following: (1) DNA repair gene: Yang *et al.* (2010) have found XPC (xeroderma pigmentosum disease gene C) in bladder cancer tissues hypermethylation extent than normal mucosa high, XPC gene defects and high-level bladder cancer, also found that patients with bladder cancer survival rate compared with XPC defects no defects were low. (Hauser *et al.*, 2013) and so on 227 patients (non-muscle invasive bladder cancer, N = 75; muscle invasive bladder cancer, N = 20; transurethral resection of bladder tumor (TURBT) without bladder cancer, N = 48; benign lesions, N = 31; healthy individuals, N = 53) in serum-related gene

methylation assay and found that patients with bladder cancer GSTP1 (glutathione S-transferase P1) and other genes are different degrees of methylation. (2) tumor suppressor gene, other related studies (Gao *et al.*, 2012) for meta-analysis to bladder cancer tumor suppressor gene RASSF1A hypermethylation and bladder cancer staging and grading of the relationship, a total of 10 eligible studies involving 543 cases and 217 controls pooled analysis. Fixed effects model, RASSF1A gene methylation or values (in bladder cancer patients compared with normal controls) was 8.40 (95% CI = 4.96-14.23); in the random effects model, tumor stage and grade or values (RASSF1A gene methylation in patients with and patients without methylation compared) to 0.75 (95% CI = 0.28-1.99) and 0.39 (95% CI = 0.14-1.09). The results show that, RASSF1A gene methylation appears to be an independent bladder cancer harbinger factor. (3) cell adhesion and migration genes, through the 98 patients with bladder cancer were detected CDH1 gene methylation, the study found 35 positive patients compared with 63 negative patients survival survival rate ($p = 0.003$), proposed CDH1 isogenic high methylation and low survival rate of patients with bladder cancer and are noted verify methylation of these genes in bladder cancer progression, diagnosis and survival relations will contribute to the clinical decision-making and individual therapy (Kandimalla *et al.*, 2013; Maruyama *et al.*, 2001). (4) cell cycle and apoptosis-related genes, Jablonowski *et al.* (2011) showed that 42 cancer patients in both the degree of methylation were: DAPK 27/42 (64.3%) and P1617/42 (40.5%), of which 12 patients were detected in both the promoter methylation in the control group, no a glycosylation. The study also found that low levels of bladder cancer were more frequently methylated DAPK and pointed out the blood of both methylation detection of bladder cancer patients may provide an effective means of early diagnosis. By methylation-specific real-time quantitative polymerase chain reaction, for 108 non-muscle invasive bladder cancer tissues, corresponding normal mucosa and urine samples BCL2 (apoptosis-related regulatory genes), hTERT (human telomerase reverse transcriptase) for methylation detection, while the 105 normal control patients accordingly and the results show Bcl2 and hTERT gene methylation levels with tumor grade and stage was positively correlated with patient age was a significant positive correlation (respectively $p = 0.004$ and $p = 0.027$) (Vinci *et al.*, 2011).

Bladder cancer gene hypomethylation: Prevalence of the entire genome of tumor cells hypomethylation, before the tumor is not malignant methylation changes have

occurred and by the metabolism may be released. In some precancerous lesions in DNA hypomethylation widespread, such as bladder cancer methylation also occur in the early stages of cancer. Research also found that hypomethylation of DNA exists in bladder cancer and loci CpG of non-CpG island and CpG islands and in urothelial cell carcinoma by a higher level by the low level of DNA hypomethylation state more significant (Wolff *et al.*, 2010). Using MSP analysis revealed that 59.26% (16/27) of bladder cancer HPSE (heparanase) gene hypomethylation changes occurred, while in normal bladder tissue hypomethylation proportion was only 20.00% (3/15), the difference was statistically significant ($p < 0.05$), inferred HPSE gene promoter hypomethylation can lead to over-expression of HPSE, resulting in the occurrence of bladder cancer and play a role in the development process. Wilhelm *et al.* (2010) found that bladder LINE1 hypomethylation and susceptibility to bladder cancer, bladder cancer can be used as diagnostic and therapeutic potential biomarkers.

PROSTATE CANCER GENE METHYLATION

DNA methylation has much biological significance, the normal methylation can maintain the body's normal function and hypermethylation easily lead to disease and even cancer. Prostate cancer related gene DNA methylation also include: tumor suppressor gene hypermethylation and hypomethylation of genomic breadth.

Prostate cancer gene hypermethylation: Recent studies have found that many of the genes in prostate cancer at high CpG island methylation status and this led to hypermethylation promoter gene silencing and inactivation and the relevant silent prostate cancer genes are involved in DNA damage repair, tumor cell invasion/metastasis, cell cycle regulation and other processes. Common prostate cancer -prone DNA methylation of genes mainly include the following: (1) DNA damage and repair related genes, GSTP1 is the most common prostate cancer gene hypermethylation in prostatic intraepithelial neoplasia were also seen, but rare in benign prostatic disease (Ellinger *et al.*, 2011); Yoon *et al.* (2012) through pyrosequencing tissue in 100 patients (55 cases and 45 cases of prostate cancer, benign prostatic hyperplasia) in GSTP1 methylation detection and its methyl level and pathological parameters were analyzed and found GSTP1 methylation levels in prostate cancer samples was significantly higher than that BPH samples ($56.7 \pm 32.7\%$ higher than $1.6 \pm 2.2\%$, $p < 0.001$), GSTP1 methylation and cancer of the prostate and

PSA the level, but the methylation levels with age, Gleason score and pathological stage no correlation. (2) tumor cell invasion / metastasis associated genes, CDH1 (E-cadherins, E-cadherin), a role in cell-cell adhesion molecules, the calcium ion binding site, the maintenance of calcium ions through the epithelial cells complete the form and structure play an important role. Related research through pyrosequencing of prostate cancer and benign prostatic hyperplasia tissue paraffin specimens such quantitative detection CDH1 gene promoter methylation status and found that prostate cancer and benign prostatic hyperplasia compared CDH1 gene hypermethylation was 32 % (8/25) and proposed other CDH1 gene can be used as an early diagnosis of prostate cancer molecular markers. (3) Tumor suppressor factor, 106 cases of benign prostatic hyperplasia and 112 cases of prostate cancer patients APC methylation detection and APC methylation levels and clinicopathological parameters of the relationship between discussed, APC methylation levels in prostate cancer specimens were significantly higher than BPH specimens ($33.3 \pm 20.7\%$ over $1.3 \pm 1.8\%$, $p < 0.001$). APC methylation level was positively correlated with Gleason score (trend $p = 0.016$), but with PSA levels or no association between installments, the findings show that, APC methylation associated with prostate cancer and invasive (Yoon *et al.*, 2013). (4) Hormone responsive genes, the study found in androgen-dependent prostate cancer, AR gene deletion and promoter hypermethylation, whereas the expression of AR loss can lead to androgen-independent prostate cancer, the normal AR gene was transfected into androgen-independent prostate cancer cells can restore their hormone-dependent, anti-androgen therapy to regain sensitivity. (5) Signal transduction genes, RASSF 1 as Ras effector protein kinase MTS1 combined with proapoptotic promote apoptosis CyclinD1 can inhibit the accumulation of the cell cycle arrest at the G1/S phase, now that the gene promoter promoter methylation causes inactivation of its expression, the related study found that the presence of prostate cancer RASSF 1 hypermethylation, also found its methylation frequency in invasive low-grade malignant tumors is high and prostate cancer infiltration (Park, 2010; Kawamoto *et al.*, 2007). (6) Cell cycle-related genes, Henrique *et al.* (2006) found that cyclin D2 promoter hypermethylation: Prostate cancer patients 117/118, high-grade intraepithelial neoplasia 38/38, benign prostatic hyperplasia 24/30, normal prostate tissue 11/11 and prostate cancer cell lines 4/4, the methylation levels in prostate cancer carcinoma highest prostate cancer tissue corresponding to the lowest level of mRNA expression and found that the methylation status and tumor stage and Gleason score correlation

(respectively $R = 0.29$, $p = 0.0014$; $R = 0.32$, $p = 0.0005$), proposed cyclin D2 promoter hypermethylation and tumor invasiveness.

Prostate cancer gene hypomethylation: Genome hypomethylation cancer is one of the characteristics, including oncogenes, repeat sequences and metastasis lower degree of methylation of genes. DNA hypomethylation also associated with prostate cancer progression, Wang *et al.* (2007) found that CRIP 1, WNT5A and S100P genes in the prostate apparent hypomethylation, whereas in normal prostate tissue was not found. Flori *et al.* (2004) found that 49 percent of prostate cancer appears LINE-1 hypomethylation in non-cancer samples found no significant hypomethylation phenomenon.

DNA METHYLATION AND THE DIAGNOSIS OF UROLOGICAL CANCERS

Urological cancers formation by genetic and epigenetic modifications impact and methylation is the most thorough study epigenetic mechanisms. Changes in the level of DNA methylation occurs early in the course of tumor in the urinary system and its technology is based on PCR detection method based on quite sensitive, it is an ideal biomarker.

DNA methylation and the diagnosis of renal cell carcinoma: Currently, the "kidney triad" (hematuria, flank pain, abdominal mass) clinical occurrence rate has been less than 15% of patients with these symptoms are often diagnosed with advanced (Jones and Laird, 1999). The major clinical diagnostic tools include urinary cytology, imaging and fine needle aspiration biopsy, however, low sensitivity of urine cytology, imaging studies can not accurately find some early small size kidney cancer, fine needle aspiration biopsy is a species are invasive, patients will bring pain and psychological burden, so looking for a quick, easy and non-invasive method for early diagnosis becomes particularly critical.

Organ-related tumor involving body fluids (such as urine) can detect abnormal methylation as specimens, which will give the tumor molecular detection bring great convenience, Battagli *et al.* (2003) collected 50 cases of kidney cancer patients preoperative urinary fluid, while collecting the surgical specimens examined VHL, p16/CDKN2a, p14ARF, APC, RASSF1A and Timp-3, etc. 6 methylation status of tumor suppressor genes, tumor samples detected 100% of the patients six genes abnormal methylation of genes, corresponding to 88% of urine samples of patients detected six genes in an aberrant

methylation of the control group of normal kidney tissue, urine sediment samples of six genes not found a glycosylation changes related through urine detecting methylation of tumor suppressor genes will contribute to the early diagnosis of renal cell carcinoma.

While serum tumor suppressor genes by methylation detected as early diagnosis of renal cell help. Some researchers on 33 patients with renal cell carcinoma and cancerous tissue tumor suppressor gene (DKK3, WIF1, SFRP1, SFRP2 etc.) methylation assay and found that serum and tumor tissues were detected methylation ratio 72.7%, while the control group was not detected methylation of the corresponding gene, the study also found that the frequency of methylation and tumor grade and stage were positively correlated (level, $p < 0.01$; installments, $p < 0.003$; M, $p < 0.02$), raised serum hypermethylation of these tumor suppressor genes will contribute to the early detection of renal cell carcinoma, progression and prognosis (Urakami *et al.*, 2006). Onay *et al.* (2009) on 21 patients with renal cell (normal tissue, precancerous tissue malignant tissue) in the seven kinds of tumor suppressor gene methylation status of testing and found that hypermethylation of tumor suppressor genes rate: RASSF1A (76%), p16 (80%), ECAD (42%), TIMP3 (33%) and MGMT (33%); while APC (14%) and RARbeta2 (19%) showed low methylation rate is and that these gene methylation profiling will help to determine the early kidney. Through the above-mentioned studies indicate that patients with tissue, blood and urine gene promoter methylation joint detection, early diagnosis of kidney cancer will help.

DNA methylation and the diagnosis of bladder cancer:

Bladder cancer laboratory examinations are cystoscopy and urine cytology. Cystoscopy invasion, is easy to bring patients the pain and psychological burden. Urine cytology, is although high specificity but poor sensitivity. Different organizations have different patterns of DNA methylation, which for the early diagnosis of cancer provides a certain basis, DNA methylation detection of bladder cancer with conventional inspection methods, compared with a high-throughput, high sensitivity, can earlier detect tiny tumors and even precancerous lesions. Application of RT-MSP detect bladder cancer cells in urine and NID2 TWIST1 methylation status of both genes, the results showed that the sensitivity and specificity of more than 90%, this method simply collecting patient urine for noninvasive detection methods, as an ideal detection method (Renard *et al.*, 2010). Scher *et al.* (2012) using nested methylation-specific polymerase chain reaction in the urine of bladder cancer patients BCL2, CDKN2A and NID2 methylation

testing, results show that this detection method to distinguish between bladder cancer and other genitourological cancers sensitivity 80.9%, specificity 86.4% and that this approach will help to pass the urine of bladder cancer patients to predict and monitor the disease.

Partial gene promoter methylation status and prognosis and survival of cancer patients, (Lin *et al.*, 2013) and so on through MS-PCR (methylation-specific polymerase chain reaction) on 135 patients with bladder cancer tumors and 34 normal controls patient organizations PCDH8 for methylation detection, while PCDH8 methylation status and demographic, clinical, pathological parameters and prognosis correlation analysis showed that patients with carcinoma of bladder cancer methylation rate 76 / 135 (56.3%), was not found in patients with normal bladder carcinoma PCDH8 methylation status with advanced cancer (T2-T4), high-grade tumors (G3), tumor diameter larger (greater than 3 cm) and non-papillary morphology significantly related and with a shorter survival time of patients showed that PCDH8 methylation and bladder cancer malignant behavior and poor prognosis. In tumor tissue promoter methylation status determination can help identify which patients need aggressive interventions to improve patient outcomes.

DNA methylation and the diagnosis of prostate cancer:

Currently biomarker for prostate cancer detection is mainly PSA (prostate specific antigen), but PSA has two shortcomings, one inspection results have high false positive, the second is not appropriate for cancer risk assessment. To find a new diagnosis of prostate cancer and to assess their risk screening method, we must identify new biomarkers, while clearly its nature. Tumor suppressor gene promoter DNA hypermethylation is a common tumor development early epigenetic phenomenon, which can cause the associated gene silencing.

GSTP1 (glutathione S-transferase P1,) gene is the most common prostate cancer gene hypermethylation in the early precancerous lesions such as prostatic intraepithelial neoplasia were also seen, but rare in benign prostatic disease (Ellinger *et al.*, 2008). Woodson *et al.* (2008) on 100 patients with prostate biopsy studies, through prostate massage urine samples collected after the detection level of GSTP1 hypermethylation, found that urine testing in GSTP1 hypermethylation of prostate cancer with a sensitivity of 75% and a specificity of 98%. Biopsy specimens have GSTP1 hypermethylation of prostate cancer with a sensitivity of 91% and a specificity of 88%. Therefore, GSTP1 early detection is expected to

be the best marker for prostate cancer. Studies have shown that gene promoter methylation status associated with tumor progression and with the tumor stage and metastasis and increased. Study found CD44 gene methylation status and progress of prostate cancer staging correlation between statistically significant ($p = 0.0438$), CD44 high frequency of methylation at different stages of prostate different frequencies, the B period is 37.5%, C phase 66.7%, D of 80% (Kito *et al.*, 2001). Therefore, the CD44 promoter methylation status monitoring helps to analyze and judge the progress of prostate cancer.

Moreover, given the complexity of the biological characteristics of the tumor alone, the determination of a particular marker is difficult to make a definitive diagnosis can be combined detection of a variety of tumor markers to improve diagnostic accuracy. Hoque *et al.* (2005) on 9 gene hypermethylation status tested and found four genes (GSTP1, ARF, p16 and MGMT) joint diagnosis of prostate cancer with a sensitivity of 87% and a specificity of 100%, while the GSTP1 alone detection sensitivity of 48% and a specificity of 100%.

DNA METHYLATION AND THE TREATMENT OF UROLOGICAL CANCERS

DNA methylation is an important epigenetic modification, but it is reversible, so the tumor or precancerous lesions treated by demethylation of gene expression can be restored, so as to achieve the purpose of prevention and treatment of cancer. From normal human cell gene CpG island methylation control, so does not affect the inhibition of DNA methylation in gene expression in normal cells. Caused by abnormal methylation of genes inactivated methylation inhibitors are very sensitive, easily re-activated for the demethylating state, so as urinary tract tumors demethylation therapy provides a theoretical basis. DNA methylation and the treatment of renal cell carcinoma

Current methods for the treatment of kidney cancer surgery, it is not sensitive to chemotherapy, hormonal therapy and no definite effect on the kidney. Immunotherapy and more used in patients with advanced kidney cancer, the efficacy is limited, the reaction rate is only 10 to 15% (Walsh *et al.*, 2003; Amato, 2000). With 5-aza-deoxycytidine (5-Aza-CdR), represented by the class of nucleoside methyl transferase inhibitor, has been widely used in reversing hypermethylation of tumor cells, the re-expression of genes inactivated, so as to achieve the effect of killing tumor cells. In recent years, 5-aza- deoxycytidine has also

been applied to the treatment of kidney cancer. The results (Kagara *et al.*, 2008) showed that people with untreated renal cancer cell lines, compared with 5 a Aza-CdR treatment, with tumor suppressor function carboxyl terminal hydrolase 1 (ubiquitin carboxyl-terminal hydrolase L1, UCHL1) expression was increased by 3.41 times; addition, 5-AZA-CdR also increase the effects of other anticancer treatments.

DNA methylation and the treatment of bladder cancer:

The clinical treatment of bladder cancer mainly surgical treatment, with drug intravesical therapy, thermotherapy, photodynamic therapy, but various treatments have some side effects and recurrence rates, such as the common tumor recurrence, cancer cells transfer and chemical cystitis. Treatment of bladder cancer is more complex, how to choose the best treatment options for clinicians must consider. In recent years, with the right DNA methyltransferase activity and DNA methylation status changed to explore the deepening of the prevention and treatment of bladder cancer is expected to provide a new strategy. Cytosine nucleoside analogues: 4-aza deoxycytidine (5-Aza-CdR) can be used as an inhibitor of DNA methylation in DNA replication process can be combined to form covalent complexes DNMT, inhibit the enzyme methyltransferase activity, to demethylation restore multiple inactivation due to methylation of tumor suppressor gene activity (Wolff *et al.*, 2010). Zhang *et al.* (2013) through methylation specific PCR detection of bladder cancer cell line T24 in Cyclin D1, VEGF-C high-methylated, treated with different concentrations of 5-Aza-CdR T24 cells were treated and then by PCR and western blotting detection of mRNA and protein expression levels were increased in research showed through a 5-aza- deoxycytidine inhibit DNA methylation in gene expression can be restored, which for the treatment of bladder cancer provide new ideas. Other studies (Christoph *et al.*, 2006) on bladder cancer cell lines display, DNMT inhibitors can reactivate APAF-1, DAPK-1 was hypermethylated gene silencing. 5-aza-deoxycytidine can effectively remove cells DNA CpG island methylation, bladder cancer treatment provides a new approach.

DNA methylation and treatment of prostate cancer:

Prostate cancer (PCa) treatment methods include surgery, chemotherapy, radiation therapy and endocrine therapy. Radical prostatectomy is mainly used in the treatment of early-stage prostate cancer patients, while a pelvic lymph node metastasis, we should not take radical surgery. Deprivation therapy is often used testicular resection, not all cancer cells apoptosis, there are androgen-independent cells survive, so only temporarily alleviate castration,

about 10-20 % of patients survive five years. This method is also easy to bring patients psychological burden (Garnick (1997)). Chemotherapy for prostate cancer has destroyed killing effect, but the effect is limited, but also damage normal cells, causing side effects. Endocrine therapy is mainly to reduce the concentration of androgen, but this method is likely to cause up-regulation of androgen receptor, resulting in prostate cancer recurrence and progression to androgen-independent prostate cancer, to the subsequent treatment of difficult. As CpG island methylation causes transcriptional inactivation of tumor suppressor genes is a reversible process, so by demethylating drugs can reverse the process and restore the function of tumor suppressor genes. Hagelgans *et al.* (2013) through a 5-aza- deoxycytidine on DU-145 cells demethylation treatment and found that it increased DU-145 cells PAI-1 (plasminogen activator inhibitor-1) of transcriptional level and the restoration of PAI-1 expression induced by cytokines. Aza -related research on hormone-independent PCa cell lines DU145 (AR gene expression was negative) for processing and found that these cells resumed androgen sensitivity (Perry *et al.*, 2006). Aza also found that other drugs may enhance the anti-tumor effect. (Fang *et al.*, 2004) found through research, Aza alone does not make DU145 apoptosis, but in combination with cisplatin may act synergistically, resulting in a large number of apoptotic cells. Therefore demethylation drugs in prostate cancer treatment have broad application prospects.

CONCLUSION

These data show that a large number of studies, DNA methylation is a tumor of the urinary system is a common feature. DNA methylation often occurs early in the urological cancers, tumor tissue through the patient, serum and urine samples in the methylation status of specific genes may be useful for screening for the early diagnosis of urinary tract tumors. Currently available for DNA methylation analysis and diagnosis of urological cancers have value target is still very small. However, the level of analysis of genome-wide DNA methylation status of the technology to improve will effectively promote DNA methylation biomarker discovery (Esteller, 2007).

DNA methylation is a reversible process, the application of DNA methylation inhibitors can make some important gene expression restored demethylation. Currently, these drugs in 5-azacytidine and 5-aza-2-deoxycytidine have been applied to clinical trials as a supplement conventional chemotherapy for the treatment of childhood leukemia and other tumors

(Whitman *et al.*, 2005). Similarly, the clinical treatment has been applied Aza leukemia, myelodysplastic syndrome and prostate cancer reported such epigenetic drug use treatment of tumors of the urinary system has broad application prospects (Ruter *et al.*, 2004).

As human epigenome program (Human Epigenome Project, HEP) start, closely associated with tumor epigenetic research has entered a new stage. DNA methylation as the main contents of HEP is currently a hot topic, with the DNA methylation detection technology and research to improve the continuous development of urinary tract tumor suppressor gene methylation profiles will further be precisely description, help to reveal urinary molecular mechanisms of tumor development, is expected to better improve urinary tract cancer early diagnosis, prognosis and treatment level has important and far-reaching significance. With the deepening of the study simultaneously, more methylated genes and the specificity of DNA methylation reversal effect of the drug will be found for the treatment of urinary tract tumors make an important supplement.

ACKNOWLEDGEMENT

This study is supported by the grant of the National S and T Major Special Project on Major New Drug Innovation (No. 2009ZX09103-643) National Science Foundation of China (No. 81072578) Fujian Provincial Department of Education projects (No. JA12434) and Quanzhou Science and Technology Project (No. 2012Z71). We thank Jia Liu and Xunwei Duan for generous reagent gifts.

REFERENCES

- Amato, R.J., 2000. Chemotherapy for renal cell carcinoma. *Seminars Oncol.*, 27: 177-186.
- Battagli, C., R.G. Uzzo, E. Dulaimi, I.I. de Caceres and R. Krassenstein *et al.*, 2003. Promoter hypermethylation of tumor suppressor genes in urine from kidney cancer patients. *Cancer Res.*, 63: 8695-8699.
- Cho, M., H. Uemura, S.C. Kim, Y. Kawada and K. Yoshida *et al.*, 2001. Hypomethylation of the MN/CA9 promoter and upregulated MN/CA9 expression in human renal cell carcinoma. *Br. J. Cancer*, 85: 563-567.
- Christoph, F., C. Kempkensteffen, S. Weikert, J. Kollermann and H. Krause *et al.*, 2006. Methylation of tumour suppressor genes APAF-1 and DAPK-1 and in vitro effects of demethylating agents in bladder and kidney cancer. *Br. J. Cancer*, 95: 1701-1707.

- De Martino, M., T. Klatte, A. Haitel and M. Marberger, 2012. Serum cell-free DNA in renal cell carcinoma. *Cancer*, 118: 82-90.
- Dudziec, E., J.R. Goepel and J.W.F. Catto, 2011. Global epigenetic profiling in bladder cancer. *Epigenomics*, 3: 35-45.
- Ellinger, J., D. Holl, P. Nuhn, P. Kahl and N. Haseke *et al.*, 2011. DNA hypermethylation in papillary renal cell carcinoma. *BJU Int.*, 107: 664-669.
- Ellinger, J., P.J. Bastian, T. Jurgan, K. Biermann and P. Kahl *et al.*, 2008. CpG island hypermethylation at multiple gene sites in diagnosis and prognosis of prostate cancer. *Urology*, 71: 161-167.
- Esteller, M., 2007. Cancer epigenomics: DNA methylomes and Histone-modification maps. *Nature Rev. Genet.*, 8: 286-298.
- Fang, X., C. Zheng and Z. Ij, 2004. Enhanced sensitivity of prostate cancer DUI45 cells to cisplatin by 5-aza-2'-deoxythymine. *Oncol. Rep.*, 12: 523-526.
- Florl, A.R., C. Steinhoff, M. Muller, H.H. Seifert, C. Hader *et al.*, 2004. Coordinate hypermethylation at specific genes in prostate carcinoma precedes LINE-1 hypomethylation. *Br. J. Cancer*, 91: 985-994.
- Gao, T., S. Wang, B. He, Y. Pan and G. Song *et al.*, 2012. The association of RAS association domain family Protein1A (RASSF1A) methylation states and bladder cancer risk: A systematic review and Meta-analysis. *PLoS One*, Vol. 7. 10.1371/journal.pone.0048300.
- Garnick, M.B., 1997. Hormonal therapy in the management of prostate cancer: From Huggins to the present. *Urology*, 49: 5-15.
- Hagelgans, A., M. Menschikowski, S. Fuessel, B. Nacke, B.M. Arneth, M.P. Wirth and G. Siegert, 2013. Deregulated expression of urokinase and its inhibitor type 1 in prostate cancer cells: Role of epigenetic mechanisms. *Exp. Mol. Pathol.*, 94: 458-465.
- Hauser, S., M. Kogej, G. Fechner, J. Von Pezold and R. Vorreuther *et al.*, 2013. Serum DNA hypermethylation in patients with bladder cancer: Results of a prospective multicenter study. *Anticancer Res.*, 33: 779-784.
- Henrique, R., V.L. Costa, N. Cerveira, A.L. Carvalho and M.O. Hoque *et al.*, 2006. Hypermethylation of cyclin D2 is associated with loss of mRNA expression and tumor development in prostate cancer. *J. Mol. Med.*, 84: 911-918.
- Hoque, M.O., O. Topaloglu, S. Begum, R. Henrique and E. Rosenbaum *et al.*, 2005. Quantitative methylation-specific polymerase chain reaction gene patterns in urine sediment distinguish prostate cancer patients from control subjects. *J. Clin. Oncol.*, 23: 6569-6575.
- Jablonski, Z., E. Reszka, J. Gromadzka, W. Wsowicz and M. Sosnowski, 2011. Hypermethylation of p16 and DAPK promoter gene regions in patients with Non-invasive urinary bladder cancer. *Arch. Med. Sci.*, 7: 512-516.
- Jemal, A., F. Bray, M.M. Center, J. Ferlay, E. Ward and D. Forman, 2011. Global cancer statistics. *CA: Cancer J. Clin.*, 61: 69-90.
- Jones, P.A. and P.W. Laird, 1999. Cancer epigenetics comes of age. *Nat. Genet.*, 21: 163-167.
- Jones, P.A. and S.B. Baylin, 2002. The fundamental role of epigenetic events in cancer. *Nat. Rev. Genet.*, 3: 415-428.
- Kagara, I., H. Enokida, K. Kawakami, R. Matsuda and K. Toki *et al.*, 2008. CpG Hypermethylation of the UCHL1 gene promoter is associated with pathogenesis and poor prognosis in renal cell carcinoma. *J. Urol.*, 180: 343-351.
- Kandimalla, R., A.A. van Tilborg and E.C. Zwarthoff, 2013. DNA Methylation-based biomarkers in bladder cancer. *Nat. Rev. Urol.*, 10: 327-335.
- Kawamoto, K., S.T. Okino, R.F. Place, S. Urakami and H. Hirata *et al.*, 2007. Epigenetic modifications of RASSF1A gene through chromatin remodeling in prostate cancer. *Clin. Cancer Res.*, 13: 2541-2548.
- Kito, H., H. Suzuki, T. Ichikawa, N. Sekita and N. Kamiya *et al.*, 2001. Hypermethylation of the CD44 gene is associated with progression and metastasis of human prostate cancer. *Prostate*, 49: 110-115.
- Laing, M.E., R. Cummins, A. O'Grady, P. O'Kelly, E.W. Kay and G.M. Murphy, 2010. Aberrant DNA methylation associated with MTHFR C677T genetic polymorphism in cutaneous squamous cell carcinoma in renal transplant patients. *Br. J. Dermatol.*, 163: 345-352.
- Lin, Y.L., J.H. Ma, X.L. Luo, T.Y. Guan and Z.G. Li, 2013. Clinical significance of protocadherin-8 (PCDH8) promoter methylation in bladder cancer. *J. Int. Med. Res.*, 41: 48-54.
- Maruyama, R., S. Toyooka, K.O. Toyooka, K. Harada and A.K. Virmani *et al.*, 2001. Aberrant promoter methylation profile of bladder cancer and its relationship to clinicopathological features. *Cancer Res.*, 61: 8659-8663.
- Morris, M.R., L.B. Hesson, K.J. Wagner, N.V. Morgan and D. Astuti *et al.*, 2003. Multigene methylation analysis of Wilms' tumour and adult renal cell carcinoma. *Oncogene*, 22: 6794-6801.
- Onay, H., S. Pehlivan, M. Koyuncuoglu, Z. Kirkali and F. Ozkinay, 2009. Multigene methylation analysis of conventional renal cell carcinoma. *Urol. Int.*, 83: 107-112.

- Park, J.Y., 2010. Promoter hypermethylation in prostate cancer. *Cancer Control*, 17: 245-255.
- Perry, A.S., R. Foley, K. Woodson and M. Lawler, 2006. The emerging roles of DNA methylation in the clinical management of prostate cancer. *Endocrine-related Cancer*, 13: 357-377.
- Pflug, B.R., H. Zheng, M.S. Udan, J.M. D'Antonio, F.F. Marshall, J.D. Brooks and J.B. Nelson, 2007. Endothelin-1 promotes cell survival in renal cell carcinoma through the ETA receptor. *Cancer Lett.*, 246: 139-148.
- Renard, I., S. Joniau, B. van Cleynenbreugel, C. Collette and C. Naome *et al.*, 2010. Identification and validation of the methylated TWIST1 and NID2 genes through real-time methylation-specific polymerase chain reaction assays for the noninvasive detection of primary bladder cancer in urine samples. *Eur. Urol.*, 58: 96-104.
- Ruter, B., P.W. Wijermans and M. Lubbert, 2004. DNA methylation as a therapeutic target in hematologic disorders: Recent results in older patients with myelodysplasia and acute myeloid leukemia. *Int. J. Hematol.*, 80: 128-135.
- Scher, M.B., M.B. Elbaum, Y. Mogilevkin, D.W. Hilbert and J.H. Mydlo *et al.*, 2012. Detecting DNA methylation of the BCL2, CDKN2A and NID2 genes in urine using a nested methylation specific polymerase chain reaction assay to predict bladder cancer. *J. Urol.*, 188: 2101-2107.
- Urakami, S., H. Shiina, H. Enokida, H. Hirata and K. Kawamoto *et al.*, 2006. Wnt antagonist family genes as biomarkers for diagnosis, staging and prognosis of renal cell carcinoma using tumor and serum DNA. *Clin. Cancer Res.*, 12: 6989-6997.
- Vinci, S., G. Giannarini, C. Selli, J. Kuncova, D. Villari, F. Valent and C. Orlando, 2011. Quantitative methylation analysis of BCL2, hTERT and DAPK promoters in urine sediment for the detection of non-muscle-invasive urothelial carcinoma of the bladder: A prospective, two-center validation study. *Urol. Oncol.*, 29: 150-156.
- Walsh, P.C., A.B. Retik, E.D. Vaughan and A.J. Wein, 2003. *Campbell's Urology*. 8th Edn., W.B. Saunders Publishing Inc., Philadelphia, PA., USA.
- Wang, Q., M. Williamson, S. Bott, N. Brookman-Amissah and A. Freeman *et al.*, 2007. Hypomethylation of WNT5A, CRIP1 and S100P in prostate cancer. *Oncogene*, 26: 6560-6565.
- Whitman, S.P., S. Liu, T. Vukosavljevic, L.J. Rush and L. Yu *et al.*, 2005. The MLL partial tandem duplication: Evidence for recessive gain-of-function in acute myeloid leukemia identifies a novel patient subgroup for molecular-targeted therapy. *Blood*, 106: 345-352.
- Wilhelm, C.S., K.T. Kelsey, R. Butler, S. Plaza and L. Gagne *et al.*, 2010. Implications of LINE1 methylation for bladder cancer risk in women. *Clin. Cancer Res.*, 16: 1682-1689.
- Wolff, E.M., H.M. Byun, H.F. Han, S. Sharma and P.W. Nichols *et al.*, 2010. Hypomethylation of a LINE-1 promoter activates an alternate transcript of the MET oncogene in bladders with cancer. *PLoS Genet.*, Vol. 6 10.1371/journal.pgen.1000917
- Wolff, E.M., Y. Chihara, F. Pan, D.J. Weisenberger and K.D. Siegmund *et al.*, 2010. Unique DNA methylation patterns distinguish noninvasive and invasive urothelial cancers and establish an epigenetic field defect in premalignant tissue. *Cancer Res.*, 70: 8169-8178.
- Woodson, K., K.J. O'Reilly, J.C. Hanson, D. Nelson, E.L. Walk and J.A. Tangrea, 2008. The usefulness of the detection of GSTP1 methylation in urine as a biomarker in the diagnosis of prostate cancer. *J. Urol.*, 179: 508-511.
- Yang, J., Z. Xu, J. Li, R. Zhang and G. Zhang *et al.*, 2010. XPC epigenetic silence coupled with p53 alteration has a significant impact on bladder cancer outcome. *J. Urol.*, 184: 336-343.
- Yoon, H.Y., Y.W. Kim, H.W. Kang, W.T. Kim and S.J. Yun *et al.*, 2012. DNA methylation of GSTP1 in human prostate tissues: Pyrosequencing analysis. *Korean J. Urol.*, 53: 200-205.
- Yoon, H.Y., Y.W. Kim, H.W. Kang, W.T. Kim and S.J. Yun *et al.*, 2013. Pyrosequencing analysis of APC methylation level in human prostate tissues: A molecular marker for prostate cancer. *Korean J. Urol.*, 54: 194-198.
- Zhang, H., F. Qi, Y. Cao, X. Zu, M. Chen, Z. Li and L. Qi, 2013. 5-Aza-2-Deoxycytidine enhances maspin expression and inhibits proliferation, migration and invasion of the bladder cancer T24 cell line. *Cancer Biother. Radiopharm.*
- Zhu, J. and X. Yao, 2009. Use of DNA methylation for cancer detection: Promises and challenges. *Int. J. Biochem. Cell Biol.*, 41: 147-154.