REVIEW ARTICLE

The study of DNA methylation in urological cancer: Present and future

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KEYWORDS
Epigenetics; DNA methylation; Methylation-specific polymerase chain reaction; Bladder cancer; Prostate cancer; Renal cancer; Testicular cancer

Abstract
Objectives: We have synthesized the principal advances in the field of the study of epigenetics and specifically DNA methylation regarding the diagnosis of urological neoplasms.
Acquisition of evidence: Review of the literature (PubMed, MEDLINE y COCHRANE) on the study of DNA methylation in urological neoplasms (prostate cancer, bladder cancer, renal cancer and testicular cancer), considering all the studies published up to January 2013.
Synthesis of evidence: It was possible to determine the state of methylation of many genes in our tumor samples. When these were compared with healthy tissue samples, it was possible to define the specific aberrant methylation patterns for each type of tumor. The study and definition of specific abnormal methylation patterns of each type of tumor are a tool having potential utility for diagnosis, evaluation, prediction of prognosis and treatment of the different forms of genitourinary cancer. The analysis of gene methylation in urine after micturition or postprostatic massage urine, semen, in the wash plasma or fluid from prostatic biopsies may allow early detection of bladder, prostate, renal and testicular cancer. In each one of the neoplasms, an epigenetic signature that may be detected in the DNA has been identified, obtained from very scarce or not at all invasive specimens, with potential in the diagnosis and evaluation of prognosis. Validation of these studies will confirm the accuracy, effectiveness and reproducibility of the results available up to now. Criteria have still not been developed that determine if a gene panel provides sufficient information in the health care practice to guide an unequivocal diagnosis or therapeutic conduct. More studies are needed to compare sensitivity, specificity, positive and negative predictive value of the test in each case. Multicenter studies analyzing the real reproducibility of these results in a clinical setting also do not exist.
Conclusions: The study of aberrant DNA methylation in biological specimens of patients has an enormous potential for the early diagnosis and screening of genitourinary neoplasms. A larger

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El estudio de la metilación del ADN en el cáncer urológico: presente y futuro

Resumen
Objetivos: Realizar una síntesis de los principales avances en el campo del estudio de la epigenética y concretamente la metilación de ADN respecto al diagnóstico de las neoplasias urológicas.

Adquisición de evidencia: Revisión de la literatura (PubMed, Medline y Cochrane) sobre el estudio de la metilación del ADN en neoplasias urológicas (cáncer de próstata, cáncer de vejiga, cáncer renal y cáncer testicular) teniendo en cuenta todos los estudios publicados hasta enero de 2013.

Síntesis de evidencia: Resulta posible determinar el estado de metilación de un gran número de genes en muestras de tumores, que al compararlo con muestras de tejido sano permite la definición de patrones de metilación aberrantes específicos para cada tipo de tumor. El estudio y la definición de patrones de metilación anómala específicos de cada tipo de tumor es una herramienta de potencial utilidad para el diagnóstico, evaluación, predicción de pronóstico y tratamiento de las diferentes formas de cáncer genitourinario. El análisis de la metilación de genes en la orina, tras la micción o masaje prostático, el semen, el plasma o el líquido de lavado de biopsias prostáticas puede permitir la detección precoz del cáncer vesical, prostático, renal y testicular. En cada una de las neoplasias se ha identificado una firma epigenética que puede detectarse en ADN obtenido de muestras muy escasamente o nada invasivas, con potencial en el diagnóstico y en la evaluación de pronóstico. La validación de estos estudios confirmará la precisión, efectividad y reproducibilidad de los resultados de los que se dispone hasta el momento.

No están aún desarrollados criterios que determinen que un panel de genes sea lo suficientemente informativo en la práctica asistencial como para guiar un diagnóstico inequívoco o una conducta terapéutica. Se requiere un mayor número de estudios para contrastar en cada caso la sensibilidad, especificidad, el valor predictivo positivo y negativo de la prueba. Tampoco existen estudios multicéntricos que analicen la reproducibilidad real de estos resultados en un entorno clínico.

Conclusiones: El estudio de la metilación aberrante del ADN en muestras biológicas de pacientes tiene enorme potencial para el diagnóstico precoz y cribado de las neoplasias genitourinarias. Se necesita un mayor número de estudios que permitan definir baterías de genes que supongan firmas inequívocas de malignidad. Esta metodología tiene también potencial a la hora de definir grupos pronóstico y potencial de respuesta a diferentes terapias.

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Introduction

All genitourinary tract cancers comprise one of the highest prevalence groups in the world. Among them, prostate cancer (PCa) is the most common solid malignancy in men, outnumbering lung and colorectal cancer cases, and it is the second most frequent cause of cancer death. Bladder cancer (BCa) is the ninth most frequent malignancy and the third leading cause of death in patients older than 80.2 Renal cell cancer (RCC) makes up about 2–3% of all adult malignancies with 20–30% of patients with metastasis at the time of diagnosis, and testicular cancer is the most frequent solid malignancy in young men.

Current methods for diagnosing and treating genitourinary tract tumors have considerably improved the prognosis and survival of patients. In particular, using PSA and biopsies for PCa, cytologies and cystoscopies for BCa and the growing number of imaging tests for renal cancer have been responsible for a migration of these diseases into early stages and a clear improvement in the prognosis in terms of survival. Nevertheless, this attitude is not without risk, and fear of overtreatment, as well as increased morbidity, represent a challenge when selecting these candidates for treatment.

Researchers have traditionally focused on studying genetic disorders (mutations, translocations, chromosomal disorders) associated with the development of cancer. Nonetheless, we now know that carcinogenesis is a consequence of both genetic and epigenetic disorders. Over recent years, interest and knowledge about epigenetic modifications associated with the development of genitourinary cancer have increased. Such advances provide an interesting scenario for early diagnosis, for knowing the prognosis of these patients and for designing the best treatment for these tumors. The possibility of determining these epigenetic modifications with minimally invasive methods, such...
as plasma, urine, saliva, and other secretion samples, could avoid the morbidity associated with currently used techniques.

There are many ways to define epigenetics; one of them is the study of changes in gene expression which are not due to changes in DNA nucleotide sequencing. The main epigenetic modifications are DNA methylation, histone post-translational modifications, and regulation of miRNA expression. Out of these, DNA methylation is the most studied one and it consists of the addition of a methyl group to the cytosines in CpG dinucleotides.

Over the last decade, a large number of studies have appeared which show the direct relationship between modifications of epigenetic mechanisms and cancer development, suggesting that these modifications would be an early and fundamental phenomenon in this group of diseases. Also, since they are potentially reversible, they could be very useful for the development of future therapeutic targets.1

Acquisition of evidence

The aim of this article is to make a synthesis of the major developments in the field of epigenetics and specifically DNA methylation regarding the diagnosis of urological neoplasms. To that end, a literature review in databases such as PubMed, Medline and Cochrane of those studies published until January 2013 was performed. The selection was limited to those articles written in English and Spanish. The search was conducted on the basis of the following terms: methylation, PCa, kidney cancer, testicular cancer, BCa, diagnosis, molecular biology, epigenetics, screening, prognosis, treatment, prevention. The methodological quality of the studies included was assessed and data extraction was carried out independently.

Synthesis of evidence

Patterns of DNA methylation

DNA methylation is the most widely studied epigenetic modification up to the present time, with a very important role in the regulation of gene expression and in the maintenance of the chromatin structure.4 In mammals, this epigenetic modification takes place at cytosines in cytosine and guanine dinucleotides (CpG). The density of this dinucleotide is very low in the genome and it is concentrated in areas that are located in the promoter regions of about 60% of known genes, constituting the so-called CpG islands.5 That methylation, which is catalyzed by DNA methyltransferase enzymes (DNMT), involves the transfer of a methyl group from S-adenosyl methionine (SAM) to carbon-5 of the cytosine. Although there are exceptions, in normal cells, CpG islands are not methylated, allowing for the expression of the gene when appropriate transcription factors are available. X chromosome inactivation in women, genomic imprinting, and the regulation of tissue-specific gene expression are among these exceptions.

A different scenario is present in tumor cells. On the one hand, there is a global loss of methylation at cytosines, and, concurrently, hypermethylation of specific genes. Hypermethylation of CpG islands located in the promoter regions of some tumor suppressor genes (TSG) prevents gene expression, and, therefore, its protective role in the development of tumors. Loss of gene expression due to hypermethylation of promoter regions affects functions which are important for the maintenance of cell homeostasis such as cell cycle, DNA repair, cellular adhesion and invasion, apoptosis phenomena, metabolism of carcinogenic agents, response to hormones, and miRNA expression, among others.6 On the other hand, there is an overall hypomethylation of the genome and a deregulation of proteins involved in the complex balance between methylation (DNMT) and the maintenance of the chromatin structure.6,7 Hypomethylation primarily affects repetitive sequences and pericentromeric regions that are methylated in normal cells. Loss of methylation at these elements in cancer may lead to the reactivation and translocation of transposons which may result in chromosomal instability and mutations caused by insertion in intergenic regions.1 These events are not only good markers for early detection of cancer, but they also increase with tumor progression.8

TSG hypermethylation is a tumor-specific epigenetic alteration.9 In the case of genitourinary cancer, among those genes inactivated due to hypermethylation of their promoter region, we find genes involved in the regulation of the cell cycle, apoptosis processes, cellular migration and invasion, among others (Table 1). Thus, characterizing methylation patterns in cancer will also allow for its use in the diagnosis and monitoring of patients. Specifically, TSG hypermethylation is a promising biomarker for several reasons. First of all, unlike mutations and other genetic alterations, methylation always occurs in defined regions of DNA and it can be detected with high specificity, sensitivity and resolution. Secondly, every tumor type has a typical methylation profile. And thirdly, methylation-specific PCR enables a fast, simple method to detect methylated alleles of a certain gene in samples with low tumor content and even in biological fluids.10

Therefore, establishing a methylation profile of each tumor type helps us classify this disease and gives us information on its prognosis. With current techniques, it is possible to quantitatively determine the state of methylation of many genes. When these were compared with healthy tissue samples, it was possible to define the specific aberrant methylation patterns for each type of tumor. The study and definition of specific abnormal methylation patterns of each type of tumor are a tool having potential utility for diagnosis, evaluation, prediction of prognosis, and treatment of cancer.

Tools for studying DNA methylation

One of the most standardized techniques to determine the presence or absence of methylation of a gene in tumor samples is methylation-specific PCR (MSP), since it requires small quantities of DNA and allows us to quickly analyze multiple markers.10 Conventional PCR cannot distinguish between methylated and unmethylated alleles, so prior to these determinations, modification of DNA with sodium bisulphite is required (unmethylated cytosines are converted into thymines by bisulphite), followed by
methylated DNA, and one methylated allele is sufficient for the activation of a methylated promoter. This makes methylated DNA an attractive target for diagnostic purposes. Furthermore, many cancers display global DNA hypomethylation, which enhances the accessibility of DNA for external modifications.

Table 1: Major genes whose methylation status is linked to various urological neoplasms. It highlights the role of these genes and their clinical useful implications.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Function</th>
<th>Neoplasia</th>
<th>Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>Cell adhesion</td>
<td>Prostate, bladder, kidney, testicle</td>
<td>Diagnosis</td>
</tr>
<tr>
<td>ARF</td>
<td>Cell cycle</td>
<td>Prostate, kidney, testicle</td>
<td>Prognosis</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Cell cycle DNA repair</td>
<td>Bladder</td>
<td>Diagnosis</td>
</tr>
<tr>
<td>CDH1</td>
<td>Cell cycle, Cellular invasion</td>
<td>Bladder</td>
<td>Diagnosis</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>Cell cycle</td>
<td>Prostate, bladder, kidney, testicle</td>
<td>Prognosis</td>
</tr>
<tr>
<td>DAPK</td>
<td>Apoptosis</td>
<td>Bladder</td>
<td>Diagnosis</td>
</tr>
<tr>
<td>SFRP</td>
<td>Cell differentiation</td>
<td>Bladder, kidney</td>
<td>Diagnosis</td>
</tr>
<tr>
<td>GATA5</td>
<td>Cell differentiation</td>
<td>Kidney</td>
<td>Prognosis</td>
</tr>
<tr>
<td>GSTP1</td>
<td>DNA repair</td>
<td>Prostate, testicle</td>
<td>Prognosis</td>
</tr>
<tr>
<td>HOX</td>
<td>Cell differentiation</td>
<td>Bladder</td>
<td>Diagnosis</td>
</tr>
<tr>
<td>HSPB1</td>
<td>Apoptosis</td>
<td>Prostate</td>
<td>Prognosis</td>
</tr>
<tr>
<td>IGFBP</td>
<td>Cell proliferation</td>
<td>Kidney</td>
<td>Diagnosis</td>
</tr>
<tr>
<td>MDR1</td>
<td>Transport of toxics outside the cell</td>
<td>Prostate</td>
<td>Prognosis</td>
</tr>
<tr>
<td>MGMT</td>
<td>DNA repair</td>
<td>Prostate, bladder, testicle</td>
<td>Diagnosis</td>
</tr>
<tr>
<td>PITX2</td>
<td>Proliferation and cell development</td>
<td>Prostate</td>
<td>Prognosis</td>
</tr>
<tr>
<td>PTEN</td>
<td>Cell cycle</td>
<td>Kidney</td>
<td>Prognosis</td>
</tr>
<tr>
<td>RAR62</td>
<td>Cell development</td>
<td>Prostate, bladder</td>
<td>Diagnosis</td>
</tr>
<tr>
<td>RASSF1</td>
<td>Cell cycle, apoptosis, microtubule stability</td>
<td>Prostate, bladder, kidney, testicle</td>
<td>Prognosis</td>
</tr>
<tr>
<td>TIMP3</td>
<td>Apoptosis</td>
<td>Kidney</td>
<td>Diagnosis</td>
</tr>
<tr>
<td>TWIST1</td>
<td>Cell adhesion</td>
<td>Bladder</td>
<td>Diagnosis</td>
</tr>
<tr>
<td>VHL</td>
<td>Apoptosis</td>
<td>Kidney</td>
<td>Diagnosis</td>
</tr>
</tbody>
</table>

Non-invasive diagnosis of urological cancer

Obtaining DNA in non-invasive ways is an ideal scenario for the detection of urological malignancies. A urine test after prostate massage, semen, plasma collection, or the analysis of prostate biopsies is needed for the early detection of bladder, prostate, kidney, and testicular cancers. Currently, the main goal is to determine the real impact of altered methylation patterns on urological cancer and its role in the diagnosis, monitoring, prognosis, and treatment of these malignancies.

Diagnosis of prostate cancer

PSA and digital rectal examinations form a basic binomial for the diagnosis of suspected PCa. The increased incidence is largely due to routine use of PSA as a marker of PCa. Taking into account that this attitude is accompanied by an increase in the number of biopsies and a greater number of patients, concern about overtreatment of this disease is explained. Besides, routine use of PSA as a marker has been questioned due to its low specificity in PCa. Tumor markers have recently been developed with the aim of improving sensitivity and specificity; examples include PCA1 and the fusion gene TMPRSS2:ERG. Nevertheless, at this moment, there is no sensitive and specific marker to aid in the diagnosis of PCa.

So far, numerous genes with abnormal methylation in PCa have been described and its possible use as markers for diagnosis has been analyzed. One of the most studied markers for the detection of PCa is the gene encoding the glutathione S-transferase pi (GSTP1), the function of which is to protect the cell from carcinogenic agents. GSTP1 is methylated
in 70–90% of tumors, but not in benign prostatic hyperplasia. Other frequently methylated genes are RASSF1A, APC and RAR2, among others. Detection of GSTP1 in urine has high specificity (87–100%), but low sensitivity (20–83%). In order to improve sensitivity in diagnoses, determining methylation in a group of genes such as [GSTP1, ARF, CDKN2A and MGMT] or [GSTP1, APC, RARB2 and RASSF1A] is found to increase sensitivity up to 86% in urine diagnosis (Table 2).

DNA methylation in the selection of patients for prostate biopsy has also been suggested. In this regard, the ProCaM prostate cancer methylation assay, a prospective study conducted in patients with PSA levels between 2.0 and 10.0 ng/ml, was proposed. Methylation of genes GSTP1, APC, and RARB2 was analyzed in post-massage urine samples, obtaining a sensitivity and specificity for diagnosis of 60 and 80% respectively. Besides, that test showed a 7.7 times increased risk of high-risk PCa in the prostate biopsy, so it may help select patients for prostate biopsy with PSA levels ranging from 2 to 10 ng/ml.

DNA methylation in PCa provides relevant information on variables associated with the prognosis for the disease. A study has recently been published which analyzed methylation of GSTP1, APC and MDR1 in 170 prostate cancer samples and compared them to 69 samples from patients with BPH. A score regarding the methylation of these 3 genes was defined, which diagnosed PCa with a sensitivity of 75.9% and a specificity of 84.1% from a specified value. In addition, this study showed that this combination of genes was related to the prognosis for the disease since it is associated with a higher T stage, high Gleason scores, and high preoperative PSA levels, so it is useful for differentiating localized disease from locally advanced disease. Recently,

### Table 2  Major studies based on the analysis of methylation patterns in different urological malignancies.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Neoplasia</th>
<th>Mean</th>
<th>Characteristics</th>
<th>Detection accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTP1, APC, MDR1</td>
<td>Prostate</td>
<td>Urine</td>
<td>–</td>
<td>75.9% sensibilidad</td>
</tr>
<tr>
<td>GSTP1, APC, RARB2</td>
<td>Prostate</td>
<td>Urine</td>
<td>–</td>
<td>84.1% especificidad</td>
</tr>
<tr>
<td>GSTP1, APC, RARB2, RASSF1A</td>
<td>Prostate</td>
<td>Urine</td>
<td>–</td>
<td>60% sensibilidad</td>
</tr>
<tr>
<td>GSTP1, ARF, CDKN2A, MGMT</td>
<td>Prostate</td>
<td>Urine</td>
<td>–</td>
<td>80% especificidad</td>
</tr>
<tr>
<td>VHL, CDKN2A, p14</td>
<td>Kidney</td>
<td>Urine</td>
<td>–</td>
<td>86% sensibilidad</td>
</tr>
<tr>
<td>APC, GSTP1, p14, p16, PTGS2, RASSF1A</td>
<td>Testicle</td>
<td>Plasma</td>
<td>–</td>
<td>89% especificidad</td>
</tr>
<tr>
<td>CDH1, CDH13, APC, RASSF1</td>
<td>Bladder</td>
<td>Fresh tumor tissue after surgery</td>
<td>High tumor grade, invasive tumor</td>
<td>–</td>
</tr>
<tr>
<td>HOXB2, KRT13, FRZB</td>
<td>Bladder</td>
<td>Paraffin-embedded tumor tissue</td>
<td>High grade, non-invasive</td>
<td>–</td>
</tr>
<tr>
<td>TWIST1, NID2</td>
<td>Bladder</td>
<td>Urine</td>
<td>–</td>
<td>90% sensibilidad</td>
</tr>
<tr>
<td>HOXA9, EOMES, ZBF154, POU4F2</td>
<td>Bladder</td>
<td>Urine</td>
<td>–</td>
<td>84% sensibilidad</td>
</tr>
</tbody>
</table>

![Image A](http://www.elsevier.es/docs/elsevier-es/...)

**Figure 1**  A. DNA after sodium bisulphite treatment: non-methylated cytosines (C) are converted into uracil, and later detected as thymine (T); the methylated cytosine in CG dinucleotide remains unchanged. B. Methylation-specific PCR analysis of amplified DNA extracted from tissue after sodium bisulphite conversion. The analysis is performed with primers for the unmethylated (U) and methylated (M) forms of the same gene in the different samples. Methylation is observed in samples 1, 4 and 6.
methylation of HSPB1 has been shown to be related to increased survival in patients with Gleason <7 (p<0.014).22 This finding may be used as an additional tool in the management of PCa patients who are candidates for active surveillance. On the other hand, methylation of PITX2 identified those patients at increased risk for biochemical recurrence after radical prostatectomy.23

**Bladder cancer diagnosis**

BCa is the second most commonly occurring genitourinary tumor and the seventh most prevalent malignancy worldwide.24 About 70% of diagnosed patients will develop this disease confined to mucus membranes. The risk of recurrence at five years for these patients, classified into groups, is 31–78% and the risk of progression to muscle-invasive disease at five years is as high as 45% in high-risk patients.24 Patients with muscle-invasive disease have a poor prognosis in spite of radical treatment. The diagnosis and follow-up of BCa are currently based on cystoscopy and urine cytology. The low sensitivity of cytology to detect low-grade tumors, about 50%,25 has been the reason why new non-invasive methods for the detection of urothelial carcinoma have been studied. Nevertheless, they have not been able to replace cytology so far.26

The presence of methylation of a single gene with enough sensitivity and specificity to help diagnose the disease has not been described in BCa. Nonetheless, methylation of a large number of genes, which play important roles in DNA repair, differentiation, apoptosis, adhesion or in the cell cycle (Table 1), has been described. Among the most frequently studied genes in the diagnosis of BCa are CDH1, APC, RASSF1A, RARB, MGMT, CDH1, GSTP1, DAPK, BRCA1, BLC2, p16, WIF1, HOX9 or the SFRP family, among others which show a sensitivity of 91% for the diagnosis of BCa in urine samples.27,28 In a recent study, methylation of TWIST1 and NID2 genes allowed for the detection of BCa in urine with a sensitivity of 90% and specificity of 93%.29 Methylation of 2NF154, POU4F2, HOXA9, and EOMES enabled the detection of BCa in urine with a sensitivity of 84% and specificity of 96%.30

Methylation of certain genes in BCa has also shown some relationship with prognosis. In particular, the study of methylation of CDH1, CDH13, APC and RASSF1A in 98 TUR specimens or cystectomy was significantly associated with a higher T stage, invasion and higher tumor grade. In addition, methylation of CDH1 was independently associated with lower survival rates in the multivariate analysis (HR: 3.3; IC 95%: 1.2–9.11).31

There are currently few tools that enable us to identify patients with BCa at high risk for infiltrating disease and who require early, aggressive treatment, as well as markers of response to treatment with agents such as BCG to determine the best treatment in those cases. Recently, simultaneous methylation of HOXB2, KRT,13 and FRZB was associated with high-grade superficial BCa (p<0.01) and HOXB2 was independently associated with infiltrating bladder cancer.35 The methylation of 25 tumor suppressor genes in patients with T1G3 (treated with BCG) was assessed in another study; this allowed for the identification of new methylated genes in this subgroup of patients. Besides, the combination of methylation of the selected genes, such as MSH6 and THBS1, may be useful for the prediction of progression and, in the near future, for making the choice of early radical treatment easier in those patients.33

**Kidney cancer diagnosis**

RCC accounts for 2–3% of all adult malignancies, its diagnosis is incidental in most cases and up to 20% of operated masses were benign ones.34 The most common histologic types of kidney cancer are clear-cell sarcoma (70%), papillary carcinoma (10–15%), and chromophobe carcinoma (5%). Sensitive and specific molecular markers are currently unavailable for the diagnosis of RCC.

Several genes with aberrant methylation that could be useful for the diagnosis of RCC, both in the familiar and sporadic forms, have been described. One of the first examples was VHL silencing via promoter methylation.35 Afterwards, methylation of numerous TSGs, such as RASSF1A, TIMP3, IGFBP1, IGFBP3, UCHL1, KTN19, BNC1 or SRFP among others, was described.36,37 Methylation of some of these genes has also been detected in urine and plasma samples. In this regard, it was possible to detect methylation of VHL, p16/CDKN2a, p14ARF, APC, RASSF1A and TIMP3 in the urine of 88% of patients with RCC and not in healthy individuals or in patients with benign kidney diseases,38 thus making it possible to develop in a short time gene panels for early detection of this malignancy.

With regard to prognosis, Wnt-antagonist family genes were associated with a higher tumor grade and more advanced stages of the disease,39 and methylation of HIC1 or GATA5 was associated with a worse prognosis in terms of survival in patients with RCC.40,41 Other genes which are associated with a poor prognosis are EPB41L3, JUP, PTEN, APAF or DAPK.42

**Testicular cancer diagnosis**

As in all other tumors, methylation of numerous genes (RASSF1A, APC, MGMT, hMLH1, RARB2 or VGF) has been described in testicular cancer. Interestingly, there are differences between methylation in seminomatous tumors and non-seminomatous ones, as evidenced by a higher percentage of methylation of MGMT, VGF, ER-β and FFBP4 in the latter group of tumors.43 The analysis of methylation in plasma of patients with testicular cancer established that the panel comprised by APC, GSTP1, P14, P16, PTGS2, and RASSF1A detected this type of tumors with a sensitivity of 75% and specificity of 80%.44 On the other hand, the normally methylated 5’ end of the XIST gene, which maps to the X chromosome, is hypomethylated in blood samples in 64% of testicular tumors.45 Although further studies are needed, these results suggest that hypomethylation of the XIST gene may be useful for the diagnosis of testicular cancer.

**Conclusions**

A large number of studies show promising results with regard to the detection of methylated genes and new challenges at clinics. In BCa, methylation of GSTP1 has become an
increasingly important tool for diagnosis, both in urine and even in flushing fluid from prostate biopsy samples. The detection of genes associated with a higher risk of biochemical recurrence or a poor prognosis is equally promising for the management of the disease. In those patients with hematuria, the detection of abnormal methylation patterns may help determine if the patient suffers from genitourinary cancer, thus, it may be useful for non-invasive screening and follow-up. The study of methylation patterns in RCC may improve early diagnosis of this malignancy, and it may even be a useful tool for follow-up purposes and when giving advice about optimal antiangiogenic therapy, or when suggesting active surveillance of the disease. However, criteria have still not been developed that determine if a gene panel provides sufficient information in the health care practice to guide an unequivocal diagnosis or therapeutic conduct. Therefore, more studies are needed to compare sensitivity, specificity, positive and negative predictive value of the test in each case. Multicenter studies are also needed to analyze the real reproducibility of these results in a clinical setting.

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**Conflict of interest**

The authors declare that they have no conflict of interests.

**References**


