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POTENTIAL NEW MARKERS IN THE EARLY DETECTION OF BLADDER CANCER

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Abstract

Purpose of Review—Bladder cancer remains a highly prevalent and lethal malignancy. Early diagnosis and prompt treatment have been shown to improve survival at both initial diagnosis and recurrence. A vast number of tumor markers have been identified and rigorously evaluated in attempts to improve non-invasive diagnostic accuracy of bladder cancer. Hematuria was the first tumor marker in a field that has grown to include soluble markers, cell-surface antigens, cell-cycle related proteins, and genetic alterations. We aim to provide a critical appraisal of newer markers and the current state of research.

Recent Findings—The number of tumor markers identified has been exponentially increasing. For a variety of reasons, many are unsuitable for clinical practice. More promising recent markers include those discovered in the fields of genomics, proteomics, and epigenetics. Much of the recent work is focused on molecular genetic pathways in bladder cancer.

Summary—The field of bladder cancer tumor markers remains a rapidly evolving area in which newer markers are constantly identified, evaluated, and often discarded if they do not add significantly to the urologists' armamentarium. Newer markers rely on genetic rearrangements, molecular changes, and cell-cycle related proteins. Work is currently being done to identify the most promising markers.

Keywords

Bladder cancer; tumor markers; molecular diagnostics

INTRODUCTION

The National Cancer Institute has recently estimated that over 68, 800 new cases of bladder cancer will be diagnosed in the USA in 2008, with over 14,000 people dying of the disease [1]. Despite significant advances in treatment, including surgical techniques and adjuvant therapies, bladder cancer continues to be an extremely common disease with high mortality. Widespread screening has been shown to decrease mortality from bladder cancer [2]. However, the adoption of such programs has been slow and currently there is no widespread mechanism for screening high risk patients in use. Currently, hematuria remains the most common

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presenting symptom in bladder cancer and should precipitate urologic evaluation [3,4]. Such an assessment in high risk patients currently includes cystoscopy, cytology, and upper tract radiographic imaging such as CT scan or ultrasound. Furthermore, patients with a known history of bladder cancer must undergo extensive surveillance in many cases, consisting of repeated cystoscopies and cytological examinations. Given the cost, expertise required, and invasive nature of such diagnostic and surveillance techniques, the field of bladder tumor markers has grown significantly. The International Consensus Panel on Bladder Tumor Markers outlined a number of attributes of an effective tumor marker, including technical simplicity of detection, high sensitivity and specificity, and reliability [5]. From the first tumor marker, hematuria, to the current research into molecular genetics, new markers are frequently being discovered and refined. Several of these have been extensively tested in human trials with varying degrees of success. While a number of markers show promising results in trials, clinical acceptance has been slow. Part of this may be due to patient reluctance to rely on a non-invasive test when conventional wisdom dictates that visual analysis is most accurate. However, prior research has shown that when sensitivity is very high, patients are more willing to rely on tumor markers in place of cystoscopy [6]. Much of this research has been succinctly reviewed reported in a number of reviews of this topic which have encapsulated the field succinctly [7–11].

Previously reported categories of tumor markers, including soluble antigens, cellular morphology, and cell-surface antigens [10] continue to be heavily investigated. Well known soluble antigen markers include the BTA-Stat and BTA/TRAK group, NMP-22, survivin, HA-Hase, BCLA-4 and survivin [12]. Such markers do not need malignant urothelial cells present in a sample for evaluation. Cellular morphology is the pathological evaluation of a urine specimen for malignancy. Such testing has been repeatedly shown to have the highest specificity, however its sensitivity lags that of other bladder cancer tumor markers and such testing requires pathologic expertise [13]. Evaluation by cell-surface antigens markers rely on the presence of urothelial cells in the specimen and the detection of malignant transformation. Examples of such changes include chromosomal changes (detected by microsatellite analysis), presence of known cell surface proteins (detected by the Ucyt and DD23 tests), and evaluation of ribonucleoprotein enzymes such as telomerases [11,12,14]. However, with recent advances in laboratory techniques including molecular genetics, cellular profiling and tissue microarrays, an extremely large number of new markers have been identified. Many can be broadly categorized into the following groups: proteomics-based markers, genomic markers, and epigenetic markers.

Proteomic Markers

Proteomics refers to the study of proteins, including structure and function using technologies such as, high resolution 2-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS) [15]. NMPs are the best examples that bladder cancer markers can be identified initially through 2-DE and then developed into conventional ELISA tests. However, this proteomic technique is technical skill, its reproducibility is moderate and it cannot detect hydrophobic proteins. The technique is also labor intensive and time consuming.

SELDI (surface enhanced laser desorption/ionization) is a preferred MS-technique used to identify and quantify biomarkers form complex biological fluids such as urine and plasma. Samples are initially subjected to high throughput sample preparation techniques to remove albumin and immunoglobulins. Proteins are then captured by a selective surface and the mass of each protein is measured based on its velocity through the time-of-flight analyzer, after proteins are ionized by a laser. The SELDI-TOF measures mass-to-charge ratio and quantifies the amount of each protein present in a biological sample [16,17]. The advantages of SELDI include, rapid protein profiling to screen large numbers of samples in a clinical research setting.

A disadvantage of SELDI is that it can analyze only small molecular mass proteins that can bind to specific chips. Thus many relatively higher molecular mass biomarkers are missed unless their degraded forms are unique enough to be identified by SELDI. Bladder cancer is amenable to SELDI-based biomarker development since many tumor-associated molecules are secreted in urine. From such studies, several markers have been identified but only a small number have undergone rigorous testing with clinicopathological correlation and study. Some of those will be reviewed here, including CXCL-1, TATI (tumor associated trypsin inhibitor), and MMPs (matrix metalloproteinase).

CXCL-1 is a member of the CXC chemokine family noted to be associated with tumorigenesis, angiogenesis and metastasis [18,19]. Recently, Kawanishi *et al.* evaluated proteins that could differentiate between normal controls, non-invasive, and invasive bladder cancer patients [20]. Using cell culture from known bladder cell lines and mass spectroscopic analysis, they identified CXCL1 as a protein with significantly increased expression in invasive bladder cancer. Subsequently, they evaluated its *in vivo* utility by assaying urine samples from normal individuals, patients with non-invasive tumors, and patients with muscle invasive disease using a CXCL1 ELISA. CXCL1 showed sensitivity of 70.1% and specificity of 80.6%. Using an increased concentration of CXCL1 specifically to predict muscle invasive bladder cancer, they report 90.6% specificity but 57% sensitivity. One significant drawback of their study was the exclusion of patients with >30 leukocytes per high powered field. This was done given the prior findings of increased levels of chemokines in patients with urinary tract infection [21]. Despite this drawback, this study demonstrates that proteomic techniques such as mass spectrometry are useful in identifying bladder cancer biomarkers which then can be assayed by simpler techniques (e.g., ELISA).

A second newer set of proteomic markers undergoing further testing are matrix metalloproteases (MMPs). While MMPs have been studies for many years [22,23,24], recent advances in proteomics have allowed for more specific evaluation and identification of MMP related complexes and their utility in bladder cancer. Roy, *et al.*, reported on their findings of tumor specific urine MMP complexes including the MMP-2, MMP-9/TIMP-1 complex, MMP-9 dimer, and ADAMTS-7 [25]. They report that using a combination of MMP-2 and MMP-9 dimer in multivariate regression and binary analyses, they were able statistically significantly differentiate bladder cancer from controls. However, the study compared only normal versus bladder and prostate cancer patients. Thus, the sensitivity and specificity of any MMP to detect cancer (bladder or prostate) between 75% and 80% will need to be confirmed in a larger cohort of patients that include patients with benign urological conditions.

Other authors have used similar approaches to identify potential markers. Smalley et al used an innovative approach of analyzing subcellular particles (microparticles) released by urothelial cells by massspectormetry (LC-MS/MS; Thermo Finnigan LTQ and LTQ-FT ion trap mass spectrometer) to identify eight potential biomarkers of bladder cancer [26]. These included Resistin, GTPase NRas, mucin-4, and Retinoic acid-induced protein 3. While these proteins were found to be differentially expressed in the urine of bladder cancer patients and normal individuals, the sample size was too small. Therefore, these potential biomarkers will need to be validated in larger studies.

Recent technical advances have allowed the field of proteomics to become extremely productive in identification of proteins that are assayed in higher or lower concentrations in bladder cancer patients when compared to the urine of normals. While this has created an incredibly large pool of potential tumors and therapeutic targets, these techniques as such are powerful as initial screens to identify potential biomarkers. Following initial screening, more conventional tests (e.g., ELISA, RT-PCR assay, point-of-care devices) can be developed to test the clinical utility of potential biomarkers.

Genomic Markers

Genomic refers to the study of DNA or RNA sequences and gene expression differences between tissues resulting in signature expressions for specific cancer types [27]. Using technologies such as gene microarray technology that can analyze thousands of DNA sequences very quickly, combined with tissue microarrays, which can analyze many patients rapidly, the field of genomics has identified a very long list of genetic duplications and aberrations that may play a role in tumorigenesis [28]. The newer addition to the array technology is microRNA profiling. These techniques are considered as the “shotgun approach” [29,30], they allow an unique opportunity to identify the molecular signatures specific for tumors, and more narrowly, for various grades and stages of tumors. Some of the well known genetic markers include FGFR-3 mutations, which together with p53 and retinoblastoma genes have helped identify divergent molecular pathways in the development of bladder cancer [31,32,33]. Since these markers have been the subject of various reviews, they will not be covered in this review. Newer markers detected include Aurora Kinase A (AURKA) amplification, ETS-2/uPA mRNA ratio, and a combination of markers such as RNAs CDC-2 and HOXA-13.

Aurora Kinase A is a gene encoding a key regulator of mitosis. It has been previously identified as a tumor susceptibility gene in mice studies and overexpression of this gene has been shown to promote malignant transformation and progression of mammalian neoplasms [34]. Recently, Park *et al* noted gene amplification (at least 3 to 4 copies per cell) for AURKA and utilized FISH (flourescent in-situ hybridization) on voided urine specimens to assess the number of AURKA gene copies using two probes for this target [35]/. In a case control study, involving bladder cancer, normal individuals and patients with benign conditions, they reported 96.6% specificity and 87% sensitivity for AURKA-FISH test to detect bladder cancer. They also noted that high grade tumors had increased AURKA aneuploidy than those of G1-G2; however, no differences were noted in stage. They conclude that, in this study, the AURKA FISH assay was more effective than cytology in detecting bladder cancer and this marker holds promise for the future.

Quantitative reverse transcription PCR (Q-PCR) RNA isolated from exfoliated urothelial cells in urine is a promising technique in identifying and validating new biomarkers. A number of markers have been identified but recently a very promising mRNA ratio has been reported [36]. Hanke *et al* analyzed the expression of BAX, Bcl2L1, ETS-2 (erythroblastosis virus E26 oncogene homolog 2), Ki-67, HTATIP2 (HIV-1 TAT interactive protein 2), STMN1 (Stathmin 1), UPK1A (uroplakin 1 A) and uPA mRNA by Q-PCR and normalized the expression to GAPDH. In this study, ratio of ETS-2 to uPA yielded was an independent marker for detecting bladder cancer. The ratio had about 89% sensitivity and 89% specificity to detect bladder cancer. The sensitivity for detecting low grade tumors was rather weak (~ 53%). These results point to the mRNA ratio of ETS-2 to uPA as a promising potential bladder cancer tumor marker.

Rosser et al used the cDNA microarray (gene profiling) approach to determine the molecular signatures of bladder cancer in the exfoliated cells in urine [37]. In a study of 46 individuals they identified 14 up-regulated and 10 down-regulated genes in exfoliated tumor cells. Out of these 24 differentially expressed genes, they used 14, as the molecular signature for diagnosing bladder cancer; this molecular signature had 90% sensitivity and 65% specificity. Although, this is a small study and the specificity of this molecular signature appears to be low, study is significant since it used the exfoliated cells as a source to conduct cDNA microarray analysis. This molecular signature can be validated in a larger study using Q-PCR.

Holyoake *et al* evaluated the expression of 14 different genes by Q-PCR. In their analyses they developed a combined 4-marker test involving HOX-A13, IGBP-5, MDK, and CDC-2 mRNAs (uRNA-D) for the detection of urothelial cancer [38]. The uRNA-D, at 85% specificity had

48%, 90% and 100% sensitivity to detect Ta, T1, and T2-4 tumors (respectively). As expected, the sensitivity to detect Ta tumors increased to 60% for primary Ta tumors (primary tumors are usually larger than recurrent tumors) and 76% for Ta tumors > or =1 cm in diameter. Although, the sample size is small (20 patients), the test shows ~ 80% specificity for urinary tract infections and other benign genitourinary conditions. At the present time, these markers have not been compared with other markers and will need further validation, especially if a combination of CDC2 and HOXA13 can distinguish between low- and high-grade tumors and between Ta and ≥ T1 tumors with acceptable sensitivity and specificity.

Adding to the already crowded field of DNA/RNA-based bladder tumor markers are miRNA-profiling and miRNA-based markers. microRNAs (miRNA) are single-stranded RNA consisting of 21–23 nucleotides. Within the last three years, research on miRNAs has shown that these small RNAs are a key regulators of gene expression. To date more than 800 miRNAs have been found. Each miRNA targets and controls the expression of several genes, and therefore, the understanding the function of each miRNA in normal cellular behavior and in cancer is an active area of research. Similar to cDNA microarray profiling, which through a hybridization technique analyzes the expression of over 20,000 genes at a time, miRNA profiling for the known miRNAs is a routine technique. Hanke et al examined the expression of 157 miRNAs in exfoliated cells using Q-PCR. They found that the ratio of miRNA-126: miRNA-152 has 72% sensitivity and 82% specificity to detect bladder cancer [39].

Genetic markers of bladder carcinoma have been evaluated for many years. Recent technological advances have allowed increased breadth to the field with the identification of the number of newer markers. However, such markers will not become clinically applicable until simpler detection methods exist, standardization of markers takes place, and differentiation and specific applications for primary diagnosis versus recurrent disease are made more clear [40,41].

Epigenetic Markers

Epigenetics is a field that has co-evolved with genomics and proteomics. While genomics and proteomics relate to changes in gene or protein structure or number, epigenetics refers to reversible changes in gene function that occur without any change in genetic sequence [42]. The most common epigenetic changes investigated in bladder tumor markers relate to DNA methylation, and this topic will be covered exclusively. The mechanism of DNA methylation resulting in expression changes has been well characterized. Catalyzed by an enzyme known as DNA methyltransferase, the process adds a methyl group to the cytosine ring of the CpG dinucleotide(s). These CpGs occur in the promoter regions of genes at high density (CpG-island) and their methylation results in gene silencing [43]. In normal homeostasis, DNA methylation is a necessary component of genomic stability and transcription [44]. However, hypermethylation of tumor suppressor genes (i.e., their silencing) is a common event in during cellular transformation and tumor progression [45].

A large number of genes and their methylation state as it relates to urothelial cancer have been evaluated. Methylation analysis usually involves methylation specific PCR. However, at present no standardization exists concerning which markers are assayed and in what combination [42]. In a study of 57 bladder cancer patients and 20 control individuals, Lin et al evaluated promoter methylation of E-cadherin, p16, p14, and RASSF1A in DNA isolated from exfoliated cells, by methylation specific PCR [46]. RASSF1A was reported to be the best individual marker amongst the group, with a sensitivity of 65%. When RASSF1A was combined with p14 and E-cadherin the sensitivity increased to 83%. Interestingly, they found that as the grade of the tumor increased, the detection rate decreased slightly. For low-grade and non muscle invasive tumors the sensitivity was 85%, while for high-grade muscle invasive

tumors, it was 75%. It should be noted however, that the sample size between 13 and 33 patients for various tumor categories is small and this marker will need further validation.

Yu, *et al.,* reported a larger panel of epigenetic target genes [47]. Out of a test set of over 50 genes, they found 11 genes that were significantly hypermethylated in tumor samples compared to controls. Individually, the sensitivity and specificity was poor for each target. However, taken as a group, the sensitivity rose to 91.7% and the specificity to 87.6%. They also interestingly report that the panel that most accurately detected urothelial cancer in Chinese patients was quite different from American panels, suggesting a possible underlying epigenetic disparity.

The epigenetic markers present a new paradigm in biomarker development for bladder cancer. However, as described above, these markers have been tested in single institution and small case control studies. At the present time, the markers and the methodologies to assess them have not been standardized. Thus, as with proteomic and genomic markers, epigenetic markers will have to be validated in multicenter cohort studies.

Conclusion

Bladder cancer tumor markers remains a rapidly evolving field. Newer technologies including mass spectroscopy, liquid chromatography, tissue microarrays, gene-expression profiling and epigenetic markers are creating more and more candidates each month. Here, the most recent advances and potential tumor markers have been summarized. The two main conclusions from this review are (1) there exists a dizzying number of markers identified using newer expertise, and (2) much more work will need to be done to delineate which markers may be clinically applicable and which will be discarded. An understanding of the technological basis and refinement of these markers will aid the urologist in future critical evaluation of this field.

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References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics. CA Cancer J Clin 2008;58:71–96. [PubMed: 18287387]

2*. Messing EM, Madeb R, Young T, Gilchrist KW, Bram L, Greenberg EB, Wegenke JD, Stephenson L, Gee J, Feng C. Long-term outcome of hematuria home screening for bladder cancer in men. Cancer 2006;107(9):2173–9. [PubMed: 17029275]

3*. Grossfeld GD, Wolf JS Jr, Litwan MS, Hricak H, Shuler CL, Agerter DC, Carroll PR. Asymptomatic microscopic hematuria in adults: summary of the AUA best practice policy recommendations. Am Fam Physician 2001;63(6):1145–54. [PubMed: 11277551]

4. Nieder AM, Lotan Y, Nuss GR, Langston JP, Vyas S, Manoharan M, Soloway MS. Are patients with hematuria appropriately referred to Urology? A multi-institutional questionnaire based survey. Urol Oncol. 2008 Dec 19;

5**. Lokeshwar VB, Habuchi T, Grossman HB, Murphy WM, Hautmann SH, Hemstreet GP 3rd, Bono AV, Getzenberg RH, Goebell P, Schmitz-Dräger BJ, Schalken JA, Fradet Y, Marberger M, Messing E, Droller MJ. Bladder tumor markers beyond cytology: International Consensus Panel on bladder tumor markers. Urology 2005;66:35–63. [PubMed: 16399415]

6. Yossepowitch O, Herr HW, Donat SM. Use of urinary biomarkers for bladder cancer surveillance: patient perspectives. J Urol 2007;177:1277–1282. [PubMed: 17382711]

7. Vrooman OP, Witjes JA. Urinary markers in bladder cancer. Eur Urol 2008;53(5):909–16. [PubMed: 18162285]

8. Black PC, Brown GA, Dinney CP. Molecular markers of urothelial cancer and their use in the monitoring of superficial urothelial cancer. J Clin Oncol 2006;24(35):5528–35. [PubMed: 17158538]
9. Messing E. Markers of detection. Urol Oncol 2007;25(4):344–7. [PubMed: 17628306]
10. Shirodkar SP, Lokeshwar VB. Bladder tumor markers: from hematuria to molecular diagnostics--where do we stand? Expert Rev Anticancer Ther 2008;8(7):1111–23. [PubMed: 18588456]
11. Konety BR. Molecular markers in bladder cancer: a critical appraisal. Urol Oncol 2006;24(4):326–37. [PubMed: 16818187]
12. Herman MP, Svatek RS, Lotan Y, Karakiewizc PI, Shariat SF. Urine-based biomarkers for the early detection and surveillance of non-muscle invasive bladder cancer. Minerva Urol Nefrol 2008;60(4):217–35. [PubMed: 18923359]
13. Budman LI, Kassouf W, Steinberg JR. Biomarkers for detection and surveillance of bladder cancer. Can Urol Assoc J 2008;2(3):212–21. [PubMed: 18682775]
14. Bennett A. Telomerase and other novel approaches to bladder cancer detection. Clin Lab Sci 2008;21(3):185–90. [PubMed: 18678141]
15**. Schiffer E, Mischak H, Theodorescu D, Vlahou A. Challenges of using mass spectrometry as a bladder cancer biomarker discovery platform. World J Urol 2008;26(1):67–74. [PubMed: 18175124]
16. Diamandis EP. How are we going to discover new cancer biomarkers? A proteomic approach for bladder cancer. Clin Chem 2004;50(5):793–5. [PubMed: 15105344]
17. Petricoin EF, Zoon KC, Kohn EC, Barrett JC, Liotta LA. Clinical proteomics: translating benchside promise into bedside reality. Nat Rev 2002;1:683–95.
18. Tanaka T, Bai Z, Srinoulprasert Y, Yang BG, Hayasaka H, Miyasaka M. Chemokines in tumor progression and metastasis. Cancer Sci 2005;96:317–22. [PubMed: 15958053]
19. Wang B, Hendricks DT, Wamunyokoli F, Parker MI. A growth-related oncogene/CXC chemokine receptor 2 autocrine loop contributes to cellular proliferation in esophageal cancer. Cancer Res 2006;66:3071–7. [PubMed: 16540656]
20. Kawanishi H, Matsui Y, Ito M, Watanabe J, Takahashi T, Nishizawa K, Nishiyama H, Kamoto T, Mikami Y, Tanaka Y, Jung G, Akiyama H, Nobumasa H, Guilford P, Reeve A, Okuno Y, Tsujimoto G, Nakamura E, Ogawa O. Secreted CXCL1 is a potential mediator and marker of the tumor invasion of bladder cancer. Clin Cancer Res 2008;14(9):2579–87. [PubMed: 18451219]
21. Otto G, Burdick M, Strieter R, Godaly G. Chemokine response to febrile urinary tract infection. Kidney Int 2005;68:62–70. [PubMed: 15954896]
22. Curran S, Murray GI. Matrix metalloproteinases in tumour invasion and metastasis. J Pathol 1999;189:300–8. [PubMed: 10547590]
23. Nagase H, Woessner JF Jr. Matrix metalloproteinases. J Biol Chem 1999;274:21491–4. [PubMed: 10419448]
24. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nature reviews 2002;2:161–74.
25. Roy R, Louis G, Loughlin KR, Wiederschain D, Kilroy SM, Lamb CC, Zurakowski D, Moses MA. Tumor-specific urinary matrix metalloproteinase fingerprinting: identification of high molecular weight urinary matrix metalloproteinase species. Clin Cancer Res 2008;14(20):6610–7. [PubMed: 18927302]
26. Smalley DM, Sheman NE, Nelson K, Theodorescu D. Isolation and identification of potential urinary microparticle biomarkers of bladder cancer. J Proteome Res 2008;7(5):2088–96. [PubMed: 18373357]
27. Vrooman OP, Witjes JA. Molecular markers for detection, surveillance and prognostication of bladder cancer. Int J Urol 2009;16(3):234–43. [PubMed: 19298346]
28. Lodde M, Fradet Y. The detection of genetic markers of bladder cancer in urine and serum. Curr Opin Urol 2008;18(5):499–503. [PubMed: 18670274]
29. Michiels S, Koscielny S, Hill C. Prediction of cancer outcome with microarrays: A multiple random validation strategy. Lancet 2005;365:488–92. [PubMed: 15705458]

30. Wang Y, Barbacioru C, Hyland F, et al. Large scale real-time PCR validation on gene expression measurements from two commercial long-oligonucleotide microarrays. BMC Genomics 2006;7:59. [PubMed: 16551369]

31. Mitra AP, Birkhahn M, Cote RJ. p53 and retinoblastoma pathways in bladder cancer. World J Urol 2007;25(6):563–71. [PubMed: 17710407]

32. Shariat SF, Karam JA, Lerner SP. Molecular markers in bladder cancer. Curr Opin Urol 2008;18(1): 1–8. [PubMed: 18090481]

33. Billerey C, Chopin D, Aubriot-Lorton MH, et al. Frequent FGFR3 mutations in papillary noninvasive bladder (pTa) tumors. Am J Pathol 2001;158:1955–1959. [PubMed: 11395371]

34. Ewart-Toland A, Briassouli P, de Koning JP, et al. Identification of Stk6/STK15 as a candidate low-penetrance tumor-susceptibility gene in mouse and human. Nat Genet 2003;34(4):403–412. [PubMed: 12881723]

35. Park HS, Park WS, Bondaruk J, Tanaka N, Katayama H, Lee S, Spiess PE, Steinberg JR, Wang Z, Katz RL, Dinney C, Elias KJ, Lotan Y, Naeem RC, Baggerly K, Sen S, Grossman HB, Czerniak B. Quantitation of Aurora kinase A gene copy number in urine sediments and bladder cancer detection. J Natl Cancer Inst 2008;100(19):1401–11. [PubMed: 18812553]

36. Hanke M, Kausch I, Dahmen G, et al. Detailed technical analysis of urine RNA based tumor diagnostics reveals ETS2/urokinase plasminogen activator to be a novel marker for bladder cancer. Clin Chem 2007;53:2070–2077. [PubMed: 17921261]

37. Rosser CJ, Liu L, Sun Y, et al. Bladder cancer-associated gene expression signatures identified by profiling of exfoliated urothelia. Cancer Epidemiol Biomarkers Prev 2009;18:444–453. [PubMed: 19190164]

38. Holyoake A, O'Sullivan P, Pollock R, Best T, Watanabe J, Kajita Y, Matsui Y, Ito M, Nishiyama H, Kerr N, da Silva Tatley F, Cambridge L, Toro T, Ogawa O, Guilford P. Development of a multiplex RNA urine test for the detection and stratification of transitional cell carcinoma of the bladder. Clin Cancer Res 2008;14(3):742–9. [PubMed: 18245534]

39. Hanke M, Hoefig K, Merz H, et al. A robust methodology to study urine microRNA as tumor marker: microRNA-126 and microRNA-182 are related to urinary bladder cancer. Urol Oncol. 2009 Apr 16; [Epub ahead of print].

40. Kim WJ, Bae SC. Molecular biomarkers in urothelial bladder cancer. Cancer Sci 2008;99(4):646–52. [PubMed: 18377416]

41. Lam T, Nabi G. Potential of urinary biomarkers in early bladder cancer diagnosis. Expert Rev Anticancer Ther 2007;7(8):1105–15. [PubMed: 18028019]

42. Kim YK, Kim WJ. Epigenetic markers as promising prognosticators for bladder cancer. Int J Urol 2009;16(1):17–22. [PubMed: 18721202]

43. Esteller M. Epigenetics in cancer. N Engl J Med 2008;358:1148–59. [PubMed: 18337604]

44. Baylin SB, Herman JG. DNA hypermethylation in tumorigenesis: epigenetics joins genetics. Trends Genet 2000;16:168–74. [PubMed: 10729832]

45. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 2003;349:2042–54. [PubMed: 14627790]

46. Lin HH, Ke HL, Huang SP, Wu WJ, Chen YK, Chang LL. Increase sensitivity in detecting superficial, low grade bladder cancer by combination analysis of hypermethylation of E-cadherin, p16, p14, RASSF1A genes in urine. Urol Oncol. 2009 Jan 30;

47. Yu J, Zhu T, Wang Z, Zhang H, Qian Z, Xu H, Gao B, Wang W, Gu L, Meng J, Wang J, Feng X, Li Y, Yao X, Zhu J. A novel set of DNA methylation markers in urine sediments for sensitive/specific detection of bladder cancer. Clin Cancer Res 2007 Dec 15;13(24):7296–304. [PubMed: 18094410]