REVIEW

Bladder Cancer Biomarkers: Review and Update

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Abstract

As the recurrence and mortality rates of bladder cancer are high, research is needed to find suitable biomarkers for early detection, evaluation of prognosis, and surveillance of drug responses. We performed a computerized search of the Medline/PubMed databases with the key words bladder cancer, biomarker, early detection, prognosis and drug response. Several markers were identified at DNA, RNA and protein levels with different sensitivities and specificities. Only a few of the potential bladder cancer biomarkers have been approved for clinical use. Efforts now should be concentrated on finding a panel of markers with acceptable sensitivity and specificity for early detection of bladder cancer.

Keywords: Bladder cancer - biomarker - diagnosis - prognosis

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Introduction

Bladder cancer with global annual incidence rate of 350000 (Griffiths, 2013) has gained attention within both clinicians and cancer biologists because of its recurrence and mortality rate. In the U.S.A. estimated new cancer cases and estimated deaths from it in 2013 are 72570 and 15210 respectively (Siegel et al., 2013). In Iran, bladder cancer is the fifth most common cancer among men with age-specific incidence rate of about 11.2 per 100,000 males (Karbakhsh et al., 2013). Three main subtypes of bladder cancers are transitional cell carcinoma (TCC), squamous cell carcinoma (SCC) and adenocarcinoma. TCC is the most common subtype accounting for 90% of all bladder cancer cases (Rahmani et al., 2013). At the time of diagnosis about one fifth of primary bladder cancers have invaded the muscle layer of the bladder wall and have a poor prognosis. In addition, papillary and superficial tumors recur in 70% of patients after surgical excision of tumor (Goodison et al., 2013).

At present, cystoscopy and urine cytology are recommended tools for bladder cancer diagnosis (Griffiths, 2013). However, small papillary tumors or carcinoma in situ (CIS) could be missed by standard white-light cystoscopy (WLC) so fluorescence cystoscopy as well as narrow-band imaging (NBI) cystoscopy have been developed for such cases (Cheung et al., 2013). The nature of this cancer necessitates lifelong surveillance. Consequently, bladder cancer has the highest cost from diagnosis to death among all cancers (Smith and Guzzo, 2013). This highlights the need for an appropriate cancer marker for screening of high risk people as well as surveillance of patients with history of bladder cancer. Urinary markers seem to be promising tools for diagnosis and follow-up of bladder cancer. Several molecular tests in this field have been marketed till now. Some of them are presently being used in practice, with several still under evaluation (Smith and Guzzo, 2013). As voided urine cytology (VUC) is the gold standard for the detection of bladder cancer, molecular tests are usually compared to VUC (Goodison et al., 2013). Molecular markers can be categorized to DNA, RNA, miRNA and protein markers. Few markers [fluorescence in-situ hybridization (FISH), Immuno-Cyt, nuclear matrix protein (NMP22), bladder tumor antigen (BTA) Stat, and Trak] have been approved by the US Food and Drug Administration (FDA) for use in bladder cancer surveillance and/or primary screening of high-risk people (Frantzi et al., 2012).

Literature Review

We searched the Medline/PubMed databases of the National Library of Medicine for the comprehensive information on the biomarkers introduced for bladder cancer. The terms used for the search included bladder cancer, biomarker, early detection, prognosis and drug response and the combinations of these terminologies.

Biomarker Types

DNA markers

Complex chromosomal changes as well as certain mutations have been found to correlate with different stages of bladder cancer. For instance, loss of chromosome

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9 is a very distinctive change commonly seen in Ta/T1 and less frequently in muscle invasive bladder cancers (MIBC). Since loss of chromosome 9 is recurrently seen as the single abnormality, it has been considered to be an early event in bladder cancer progression (Lindgren et al., 2006). Cytogenetic loss of chromosome 9 is demonstrated by the frequent loss of heterozygosity (LOH) on this chromosome mostly the region including CDKN2A. LOH on chromosome 9 is shown to be associated with tumor development rather than initiation (Lindgren et al., 2006).

UroVysion™: This test is aimed at detection of aneuploidy of chromosomes 3, 7, 17 and deletion of the chromosome 9p21 locus in urine specimens by FISH (Ho et al., 2013). It is among few markers achieved extensive clinical use. In comparison with urinary cytology it has more sensitivity but less specificity. It has been shown to be more powerful than cytology to diagnose stage Ta patients. It is also beneficial for monitoring patients with superficial bladder cancer after treatment with intravesical bacillus Calmette-Guerin (BCG) especially when cytology results are ambivalent (Smith and Guzzo, 2013).

Epigenetic changes

Epigenetic changes noticeably DNA methylation have extensive influence on gene expression. Recent data have indicated that aberrant DNA methylation happens commonly and early in human carcinogenesis. It has been shown to occur extensively in cancer cells and in the same promoter regions. As a result, analysis of a few loci is satisfactory for diagnosis of cancer and this is the main advantage of these markers for detection of cancer (Chihara et al., 2013). Promoter methylation has been shown to occur commonly in both normal urothelium and CIS samples from patients with urothelial carcinoma. During the progression from normal to invasive urothelial carcinoma promoter methylation is increased at both specific loci and in general. Promoter methylation seems to be a good biomarker for early detection of bladder cancer (Dhawan et al., 2006).

High throughput DNA methylation profiling in urine and tissue samples: both differential hypermethylation and hypomethylation have been seen in tumor tissues compared to normal tissues. The diagnostic accuracy of these markers in urine samples has been high, with 100% sensitivity and specificity. So according to these preliminary data, diagnostic markers based on differential DNA methylation at specific loci can be applied for non-invasive and reliable detection of bladder cancer (Chihara et al., 2013).

Detecting DNA methylation of the BCL2, CDKN2A and NID2 genes in urine: a highly specific and sensitive nested methylation specific polymerase chain reaction (PCR) assay has been developed to detect bladder cancer in small volumes of patient urine. In a pilot clinical study its sensitivity and specificity to differentiate bladder cancer from other urogenital malignancies and nonmalignant conditions have been shown to be more than 80% (Scher et al., 2012).

Detecting DNA methylation of APC, ARF, CDH1, GSTP1, MGMT, CDKN2A, RARb2, RASSF1A and TIMP3 genes in urine: the promoter methylation pattern in urine has been similar to the primary tumors. In more than two third of patients promoter methylation has been seen in at least one of these genes (CDKN2A, ARF, MGMT, and GSTP1), while none of controls have displayed such methylation. A combined two-stage predictor strategy for detection of promoter hypermethylation of these 9 genes has 82% sensitivity and 96% specificity. Therefore, quantitative methylation-specific PCR assay of a small panel of genes can be a powerful noninvasive tool for the detection of bladder cancer (Hoque et al., 2006).

Point mutations

The mutation assays are diagnostic tools to identify patients who will benefit from targeted therapies. They are also potential biomarkers to discover recurrences during surveillance of patients.

FGFR3, HRAS, KRAS, NRAS and PIK3CA mutations: in a study of mutational analysis of oncogenes performed on 257 primary bladder tumors and 184 recurrences from 54 patients, it has been suggested that surveillance by mutation analysis for FGFR3, PIK3CA and the RAS genes along with increasing the period between cystoscopies could be a practical follow-up policy for patients suffering from a non muscle invasive bladder cancer (NMIBC), grade 1-2 primary tumor. In addition, the mutation assays may be a diagnostic tool to define patients with MIBC in whom therapies targeting FGFR3 or other receptors and downstream targets may be beneficial. However, mutations in the RAS and PIK3CA genes were not predictors for either recurrence-free or disease-specific survival (Kompier et al., 2010).

FGFR3 expression and mutation analysis in cancer samples: Expression profiling, mutation analysis and LOH analysis have been used to molecularly characterize a large cohort of early-stage bladder cancer. Two types of tumors have been defined by this method. Low-grade tumors are distinguished by FGFR3 activity, either by FGFR3 mutation or by expression, high protein synthesis and low cell-cycle activity. Whereas high grade tumors show less or no dependence of the FGFR3 receptor, low levels of protein synthesis and high cell-cycle gene activity. It has been suggested that FGFR3 receptor is critically involved in low grade/stage bladder cancers (Lindgren et al., 2006).

RNA markers

Expression of Aurora-A in tissue samples: Aurora-A is an oncogenic serine/threonine kinase with critical roles in tumorigenesis as well as chemotherapy resistance. The expression levels of Aurora-A mRNA in bladder cancer tissues have been notably higher than those in adjacent non-tumor tissues (Lei et al., 2011).

Expression of p16INK4a in urinary bladder lavages: the expression of p16INK4a in cytology specimens of urine has been suggested to be a sensitive marker for urothelial carcinoma, particularly for the detection of poorly differentiated carcinomas (Tauber et al., 2011).

Expression of hTERT, SENP1, PPP1CA, and MCM5 in urine: The measurement of urinary content of hTERT, SENP1, PPP1CA, and MCM5 mRNAs has been used to identify bladder cancer recurrence. All of these mRNA markers have been shown to be more sensitive than

cytology. The combination of each marker with cytology results and also combination of hTERT and MCM5 have increased the detection rate. However, more prospective validation or registration studies should be done before these markers could enter the clinics (Brems-Eskildsen et al., 2010).

Expression of nicotinamide N-methyltransferase (NNMT) in urine and tissue samples: by comparing the gene expression profiles of tumor and normal looking tissues obtained from the same patient using cDNA macroarray, the enzyme NNMT has been identified as a highly expressed gene in bladder cancer. In addition, NNMT expression levels were notably higher in patients with bladder tumor compared to controls that showed very low or undetectable levels of NNMT expression. Urine NNMT expression levels have been suggested as a tool for early and non-invasive diagnosis of bladder cancer (Sartini et al., 2013).

Expression of cytokeratin 20 (CK20) in urine: CK20 mRNA expression has been suggested as a potential marker for detection of bladder cancer. In addition, CK20 expression has been shown to be correlated with the clinicopathologic features of bladder cancer. Besides, combination of CK20 and NMP22 has elevated the sensitivity of the method (Guo et al., 2009).

Expression of integrin-linked kinase (ILK) in tissue samples: ILK has been shown to be expressed in bladder cancer tissue significantly higher than normal adjacent tissue. Its knock down has resulted in decreased proliferation ability. So it has been suggested that ILK gene transcription and protein expression may be involved in the development of bladder cancer and it might be a novel marker for early detection of cancer and a new target for gene therapy (Wang et al., 2012).

Expression of Bmi-1 mRNA in tissue samples: expression of Bmi-1 mRNA and protein has been shown to be higher in bladder cancers than in the adjacent normal tissues. So it has been suggested as a marker for diagnosis and prognostic assessment of bladder cancer (Qin et al., 2008; 2009).

Expression of Bcl-2, caspase-3, p53, and survivin in tissue samples: these genes are involved in apoptosis and deregulation of their expression has fundamental role in carcinogenesis. Expression of Bcl-2, caspase-3, p53, and survivin has been changed in 32%, 49%, 53%, and 64% of patients respectively. Evaluation of these markers has been shown to have prognostic value in patients treated by radical cystectomy to recognize high risk patients for disease recurrence, in whom early adjuvant treatment may be required (Karam et al., 2007).

Expression of telomerase in urine: urine telomerase activity level has been shown to be an accurate marker for detection of bladder tumors (Sanchini et al., 2005). Different assay have variable sensitivities because of the low stability of telomerase and human telomerase mRNA in urine. The specificity of telomerase is notable (86.7-100%), but presence of lymphocytes may cause false positive results (Alvarez and Lokeshwar, 2007).

Expression of survivin in urine: it is a member of the inhibitor of apoptosis protein family. In a study, both sensitivity and specificity of survivin in detection of bladder cancer have been more than 80% (Srivastava et al., 2013). In a large prospective study on survivin performance for bladder cancer screening, the marker showed a good negative predictive value and specificity but a low positive predictive value and sensitivity (Johnen et al., 2012).

Expression of non-muscle myosin heavy chain IIA (NMHC IIA) in tissue samples: expression of NMHC IIA mRNA has been shown to be higher in bladder cancer compared to the corresponding normal tissues. The higher levels of its expression have been positively correlated with the histopathological classification, lymph node metastasis and mortality. High NMHC IIA expression has been shown to be an independent molecular marker (Xiong et al., 2012).

Expression of cancer-testis (CT) antigens in tissue samples: CT antigens are a group of tumor-associated antigens with restricted expression in normal tissues except for gametogenic cells but expression in a wide variety of tumors. This pattern of expression has significance in immunotherapy and early detection of cancer (Ghafouri-Fard and Modarressi, 2009; 2012). CT genes can be classified according to their locations on chromosomes to those located on X chromosome (CT-X) and those located on other chromosomes (non X-CT). Bladder cancer is among cancers with high expression of CT-X antigens(Ghafouri-Fard and Modarressi, 2009). Some CT antigens are believed to be stem cell markers(Tabarestani and Ghafouri-Fard, 2012). Expression of these antigens has been assessed in different cancers including urogenital cancers(Ghafouri-Fard et al., 2010: Ghafouri-Fard et al., 2012).

For instance, expression of six CT genes (MAGE-A3, MAGE-A1, cTAGE-1, MAGE-A12, cTAGE-2, and NY-ESO-1) has been evaluated in TCC samples. The results have shown that all of them are highly expressed in TCC but not in the adjacent normal tissues. So they have been suggested as therapeutic targets for specific immunotherapy of TCC, particularly in multi-antigen vaccine preparations (Yin et al., 2012). Another study has indicated that the most immunogenic CT antigen to date, NY-ESO-1, and/or LAGE-1 are expressed in about one third of high-grade TCCs (Sharma et al., 2003). MAGE-A10, possibly the most immunogenic antigen of the MAGE-A family has been shown to be expressed in one-third of NMIBC and its expression is correlated with high tumor grade (Mengus et al., 2013). Besides, MAGE-A4 and MAGE-A9 expressions have been demonstrated in a subset of NMIBC and MIBC, as well as CIS and lymph node metastases. MAGE-A4 and MAGE-A9 expressions have been shown to be associated with progression to MIBC with all tumors that progressed expressed MAGE-A9 (Bergeron et al., 2009). Two other CT genes, M phase phosphoprotein 1 and DEP domain containing 1 have shown critical roles in the growth of bladder cancer cells. In addition, HLA-A24-restricted peptide epitopes corresponding to parts of these 2 proteins have been identified which could induce peptide-specific cytotoxic T lymphocytes. Safety and efficacy of these peptides have been evaluated in a clinical trial (Obara et al., 2012). LY6K, another CT antigen, has been found to

be the top up-regulated one in the gene profile from the bladder cancer cell line. LY6K mRNA expression was significantly higher in bladder cancer than in normal bladder epitheliums. In addition, its knock down has resulted in significant inhibitions of cell growth, migration, and invasion (Matsuda et al., 2011). Some other CT genes such as SLCO6A1, FMR1NB and FTHL17 have been shown to be expressed in bladder cancer (Ghafouri-Fard and Modarressi, 2012).

Urothelial carcinoma-associated 1 (UCA1): it has been identified as a novel noncoding RNA gene considerably up-regulated in TCC. It has been suggested as the most TCC-specific gene identified so far. It appears to be a member of the human endogenous retrovirus H family. UCA1 assay has 91.8% specificity and 80.9% sensitivity in the diagnosis of bladder cancer (Wang et al., 2006).

microRNA (miRNA) markers

miRNAs are 18-24 nucleotide RNA molecules which are endogenous inhibitors of gene function acting by either degradation of RNA or inhibition of translation. They have been proved to be involved in pathogenesis of cancer. At present, miRNA based therapies are being investigated in cancer patients. The altered patterns of miRNAs expression may be used as novel biomarkers for early detection and therapeutic response monitoring in cancer (Nana-Sinkam and Croce, 2013). The role of miRNAs in bladder cancer development, progression and metastasis has been discussed extensively. According to reports illustrating miRNA signatures, numerous down-regulated and up-regulated miRNAs have been determined. miR-145, miR-143 and miR125b, are among those down-regulated in bladder cancer and known to be tumor suppressors, while miR-183, miR-96, miR17-5p and miR-20a are up-regulated in bladder cancer and have oncogenic properties (Yoshino et al., 2013). In an attempt to find miRNA expression profile of MIBC and NMIBC, among 15 miRNAs found to be significantly deregulated in bladder cancer, the results have been as follow: miR-146b and miR-9 were specifically up-regulated in MIBC, but others were deregulated similarly in the two types of bladder tumors: miR-200b, miR-182 and miR-138 were up-regulated while the other 10 miRNAs were down-regulated (miR-1, miR-133a, miR-133b, miR-145, miR-143, miR-204, miR-921, miR-1281, miR-199a and miR-199b)(Pignot et al., 2013). Although urinary miRNAs are suggested as potential biomarkers for bladder cancer, the main problem in this regard is that most of miRNAs are down-regulated, making it difficult to use reduced miRNAs as diagnostic markers (Kohler et al., 2013). miRNAs also appear to be engaged in cisplatin based chemotherapy response and may provide novel biomarkers for treatment response. For instance, miR-886-3p, miR923 and miR-944 have been shown to be associated with both cisplatin response and survival (Nordentoft et al., 2012).

miR-145: it has been shown to be the most recurrently down-regulated miRNA in bladder cancer which has been considerably restrained cell proliferation, migration and invasion (Yoshino et al., 2013). The sensitivity and specificity of miR-145 levels in urine to distinguish bladder cancer patients from non-cancer controls have been about 80% and 60% respectively with good correlation with grade (Yun et al., 2012).

miR-200a: it has been shown to be an independent predictor of NMIBC recurrence as a lower miR-200a level has been associated with a higher risk of recurrence (Yun et al., 2012).

miR-125b and miR-126: these 2 miRNAs have been shown to be dysregulated in the urine from cancer patients with miR-125b showing an about 10 fold decrease and miR-126 showing an about 3 fold increase in the cancer samples compared to the normal controls (Snowdon et al., 2012).

miR-200b, miR-152 and miR-10a: the expression of these miRNAs has been shown to be decreased in cancer cell lines. Besides, reduced expression of these miRNAs has been associated with increased DNA methylation in malignant cells versus primary non malignant urothelial cells. In conclusion, hypermethylation of miR-152, miR-10a and miR-200b regulative DNA sequences has been suggested as epigenetic bladder cancer biomarkers (Kohler et al., 2013).

miR-144: the expression level of this miRNA has been shown to be decreased in bladder cancer cell lines and tissues. miR-144 inhibitor has been shown to prevent the expression of endogenous miR-144 and promote cancer cell proliferation, while miR-144 over-expression has been sufficient to hamper cell proliferation. It has been suggested as an important mediator of bladder cancer cell proliferation and a novel target for anti cancer therapy (Guo et al., 2013).

miR-9, miR-182 and miR-200b: this 3-miRNA signature has been shown to be related to MIBC aggressiveness and has been associated with both recurrence-free and survival of patients (Pignot et al., 2013).

miR-126 and miR-152: the RNA ratio of miR-126 and miR-152 in urine samples has permitted the detection of bladder cancer at a specificity of 82% and a sensitivity of 72% (Hanke et al., 2010).

miR-200c, miR-141, and miR-30b: in a study aimed at identification of miRNAs functioning in the transition between the noninvasive and invasive bladder cancer, these 3 miRNAs have shown down-regulation in invasive lesions. A diagnostic test, based on these 3 miRNAs has shown a sensitivity of 100% and a specificity of 96.2% in identification of invasive cancers. Such a panel of miRNAs could identify invasive bladder tumors misclassified in pathologic evaluation of bladder biopsy specimens (Wszolek et al., 2011).

miR-135b, miR-15b and miR-1224-3p: this panel of miRNAs could detect bladder cancer with 94% sensitivity and 51% specificity and 86% concordance. miR-1224-3p alone had specificity, positive and negative predictive values and concordance of 83%, 83%, 75% and 77%, respectively. The use of this panel in patients with haematuria could detect 94% of urothelial cell carcinomas with cystoscopy rate of 26% (Miah et al., 2012).

Protein markers

A previous study aimed at establishment of a complete two-dimensional database of proteins from the urine

of patients with bladder cancer, has listed 339 proteins expressed in urine samples of bladder cancer patients with potential application as prognostic tumor markers. Of these, psoriasin, the psoriasis-associated fatty acid binding protein 5, the gelsolin fragments and prostaglandin D2 synthetase have not been described up to that time (Rasmussen et al., 1996).

US FDA has approved BTA and NMP22 for surveillance and detection of bladder cancer, respectively (Abogunrin et al., 2012). Some protein biomarkers including BTA, carcinoembryonic antigen (CEA), d-Dimer, FAS, hyaluronidase (HA), interleukin (IL)-1α, IL-6, IL-8 and vascular endothelial growth factor (VEGF) have shown significant different levels in NMIBC and MIBC. In addition, BTA, CEA, HA, IL-6, IL-8, matrix metalloproteinase-9 (MMP-9) neutrophil-associated gelatinase (NGAL) complex and VEGF levels have different levels in grades 1 and 2 compared with grade 3 tumors (Abogunrin et al., 2012).

Human complement factor H related protein: the concentration of this protein in the urine can be detected by two immunoassays named the BTA stat and the BTA TRAK tests employing the same antibody pair. The BTA stat is a qualitative test and can be done in a consultation setting while the BTA TRAK is a quantitative test performed in the laboratory (Irani et al., 1999). In a study, the BTA stat and BTATRAK tests have been shown to be better than urinary cytology in detection of bladder TCC. Nevertheless, cytology has remained the best adjunct to cystoscopy because of its high sensitivity in detection of CIS and 100% specificity (Babjuk et al., 2002). Another study has indicated that BTA TRAK is a suitable test for the diagnosis of TCC of the bladder, which is not better than urine cytology but complementary (Gibanel et al., 2002). The sensitivity of both tests in the detection of low-grade and small tumors has been low. Although these tests are most successful at detection of high grade tumors, false positives have limited their application (Alvarez and Lokeshwar, 2007).

NMP22: numerous studies have shown that increased levels of NMP22 are associated with bladder cancer. Therefore, NMP22 has been approved by FDA as a urinary biomarker. A recent study has suggested that for clinicians who would do a cystoscopy at a threshold of 5% for recurrence or 1% for progression, NMP22 level is not helpful for decision-making. However, for less risk-reluctant clinicians, NMP22 helped to show which patients needed cystoscopy and which could be spared it (Shariat et al., 2011). Systematic reviews of diagnostic value of NMP22 and urine cytology has shown that the sensitivity of NMP22 for detection of bladder cancer is higher than urine cytology, but the specificity is lower than it. So it seems that currently NMP22 cannot replace urine cytology (Hu et al., 2012). In another study, it has been revealed that UroVysion together with NMP22 can detect more cases than cytology alone, at the cost of a lower specificity. However, the increase in sensitivity is not worth the high costs of UroVysion and the false-positive tests of NMP22 (Pesch et al., 2013).

CEA: an immunofluorescence test named Immuno Cyt test has designed to detect sulfated mucin glycoproteins and CAE and has enhanced the sensitivity of cytology, especially in detection of low-grade diseases (Alvarez and Lokeshwar, 2007). CEA has been shown to be comparable with BTA and NMP22 as a single biomarker for urothelial cancer with significant elevation in smokers (Abogunrin et al., 2012).

Hyaluronic acid and hyaluronidase: an ELISA-like assay has designed to measure hyaluronic acid and hyaluronidase with 83-94% sensitivity and approximately 80% specificity. HYAL1-type hyaluronidase can also be detected at mRNA level in urine samples with 90.8% sensitivity and 93.4% specificity (Alvarez and Lokeshwar, 2007). HYAL1 levels have also correlated with tumor stage (Eissa et al., 2005).

Mcm5: increased levels of Mcm5 in urine sediments are highly predictive of bladder cancer. The sensitivity and specificity of Mcm5 test for detection of primary and recurrent bladder cancers have been 87% (Stoeber et al., 2002). The accuracy of Mcm5 immunoassay test in diagnosis of urothelial cancers is comparable with FDA-approved NMP22 ELISA Test Kit. The combination of Mcm5 and NMP22 has improved the detection of bladder cancer to 95% in clinically important cases (Kelly et al., 2012).

Tumor-associated calcium-signal transducer 2 (TACSTD2): TACSTD2 level in urinary microparticles has been shown to be associated with bladder cancer and it has been suggested as a potential biomarker for noninvasive diagnosis of bladder cancer (Chen et al., 2012).

BCLA-4: BLCA-4 is a highly specific bladder cancer marker being expressed early in the development of the disease. It is a transcription factor functioning in the regulation of the gene expression in bladder cancer (Van et al., 2004; Myers-Irvin, et al., 2005). ELISA assay aimed at detection of BLCA-4 in urine has 95-96% sensitivity and 89-100% specificity (Nielsen et al., 2006).

Cystatin B: it has been introduced as a novel tissue and urine biomarker for TCC of the bladder, with positive correlation with grade, stage, shorter time to recurrence and progression (Feldman et al., 2009).

Cytokeratins: cytokeratins are keratin-containing intermediate filaments existing in the intracytoplasmic cytoskeleton of epithelial tissue. UBC-Rapid and UBC-ELISA have been designed to detect cytokeratin-8 and -18 in the urine with different sensitivities for different grades of bladder tumors. The false positive rates of these tests and their inability to detect low-grade tumors have limited their application for bladder tumor surveillance (Alvarez and Lokeshwar, 2007).

Orosomucoid (ORM) and human zinc-alpha (2)-glycoprotein (ZAG): the levels of these proteins in urine samples have been shown to be related to the development of superficial bladder cancer and to its switch to an invasive phenotype (Irmak et al., 2005).

Reg-1 and keratin 10: The increased expression of these proteins in urine samples of patients with bladder tumors has been shown to be associated with bladder cancer progression. Reg-1 expression has associated with tumor staging and clinical outcome. In addition, Reg-1 expression could discriminate patients with bladder cancer

and controls. So it has been suggested as a biomarker for bladder cancer detection, staging, and prognosis (Orenes-Pinero et al., 2007).

Alpha-defensin: it has been recognized as a potential marker for bladder cancer in urine. In addition, alpha-defensin peptides have been shown to be commonly expressed in bladder cancer cells. So it has been suggested that autocrine tumor expression of alpha-defensins may play an important role in the invasiveness of bladder cancer in patients (Holterman et al., 2006).

Apolipoprotein A-I (APOA1), apolipoprotein A-II, heparin cofactor 2 precursor and peroxiredoxin-2: the levels of these proteins have been shown by western blot analyses to be significantly increased in bladder cancer urine specimens. The potential value of APOA1 for early detection of bladder cancer has been confirmed using a commercial ELISA test (Chen et al., 2010).

Alpha-1-antitrypsin (A1AT) glycoprotein: elevated levels of urinary A1AT glycoprotein have been shown to be indicative of the presence of bladder cancer and improved VUC results. Its sensitivity and specificity in classification of bladder cancer patients have been 74% and 80% respectively (Yang et al., 2011).

A1AT and apolipoprotein E: the combination of these 2 markers has reached 91% sensitivity and 89% specificity in detection of bladder cancer patients. These 2 markers, alone or in combination, are promising for the noninvasive detection of bladder cancer (Urquidi et al., 2012b).

VEGF: the urinary concentrations of VEGF, carbonic anhydrase IX (CA9), angiogenin (ANG), and BTA have been shown to be significantly increased in patients with bladder cancer. The sensitivity and specificity for VEGF (83% sensitivity and 87% specificity) surpassed those for BTA (80% sensitivity and 84% specificity). VEGF has been the most accurate urinary biomarker among them and has been suggested as a valuable addition to voided urine sample analysis for bladder cancer diagnosis (Urquidi et al., 2012a). Over-expression of VEGF along with survivin has been associated with a higher risk of bladder cancer recurrence (Sun et al., 2013).

C-C motif chemokine 18 (CCL18), Plasminogen Activator Inhibitor 1 (PAI-1) and CD44: urinary concentrations of CCL18 and PAI-1 have been shown to be significantly increased in bladder cancer patients. CCL18 has been the most accurate biomarker and an independent predictor of bladder cancer in voided urine samples. The combination of CCL18, PAI-1 and CD44 improved the detection of bladder cancer compared with the individual biomarkers or BTA (Urquidi et al., 2012c).

Tat-interactive protein 60 (TIP60) and meiotic recombination 11 homolog (MRE11): defects in DNA-damage response have been shown to be associated with good survival after chemotherapy and radiotherapy in previous studies. In a recent large cohort study, TIP60 and MRE11 protein expressions have been shown to be predictive markers for disease-specific survival (DSS) after cystectomy and DSS after radiotherapy respectively. Therefore, combined use of TIP60 and MRE11 has been suggested to assist treatment decisions to either cystectomy or radiotherapy (Laurberg et al., 2012).

Serine peptidase inhibitor Kazal type 5 (SPINK5), ADAM metallopeptidase domain 28 (ADAM28) and phosphotyrosine-specific protein phosphatase 1 (PTP1): these three proteins have shown significant differential expression in bladder cancer patients compared with the control group. Among them ADAM28 may serve as a possible biomarker of bladder cancer (Tyan et al., 2011).

Soluble Fas (sFas): it has been shown to be made and released by bladder TCC cells. Urine sFas is an independent marker for detection of bladder cancer recurrence and invasiveness in patients with a history of NMIBC, and it is better than NMP22 (Svatek et al., 2006).

Serine protease HtrA1: HtrA1 protein is shown to be produced by bladder urothelium in both physiological and inflammatory conditions, while it is not demonstrable in urothelial cancer cells irrespective of tumor grade and stage. However, HtrA1 mRNA levels were similar in normal and cancerous tissue. One HtrA1 isoform has been shown to be considerably down-regulated in neoplastic tissue, but significantly higher amounts of both HtrA1 forms has been detected in urine from cancer patients compared with both healthy people and patients with cystitis. HtrA1 has been shown to be a down-expressed marker since an early stage of bladder cancer development. In addition, urinary HtrA1 protein has been suggested as an early and highly sensitive and specific biomarker for bladder cancer with more than 90% sensitivity and specificity (Lorenzi et al., 2013).

CD147, Transforming growth factor beta-induced (TGFBI, BIGH3) and stathmin 1 (STMN1): BIGH3 levels have been shown to be significantly increased in the serum and urine of TCC patients. In addition, urine CD147 concentration and serum STMN1 concentration have been increased in TCC patients as compared to the controls. mRNA expressions of all molecules have been elevated in MIBC. A significant increase has been detected for STMN that correlated with disease severity. Among them, STMN1 has been suggested as the best possible diagnostic marker (Bhagirath et al., 2012).

IL-8, MMP-9 and 10, PAI-1, VEGF, ANG, CA9 and APOE: the urine concentrations of this panel of biomarkers have been measured in bladder cancer patients and controls. Seven of them show significant increase in subjects with bladder cancer relative to those without bladder cancer. The combined biomarker model has 74% sensitivity and 90% specificity. Both sensitivity and specificity have been more than urine cytology and the UroVysion cytogenetic test in this cohort (Rosser et al., 2013).

APOE, fibrinogen β chain precursor (FGB), leucine-rich α2-glycoprotein (LRG1), polymerase (RNA) I polypeptide E (POLR1E), A1AT and topoisomerase 2A (TOP2A): these proteins have been shown to be expressed in bladder cancer tissues. Elevated expressions of APOE, FGB and POLR1E have been shown to be correlated with increased tumor stage. In addition, expression of SERPINA1 in Ta and T1 tumors has been correlated with the risk of tumor progression (Linden et al., 2013).

Uroplakin 3A (Upk3A): the urinary UPK3A concentrations are consistently elevated in bladder cancer patients compared with normal volunteers with sensitivity and specificity more than 80% which outperforms

cytology and NMP22 tests. So urine measurement of UPK3A has been suggested as a sensitive marker for bladder cancer detection (Lai et al., 2010).

CD10 and the adhesion molecule E-cadherin (E-cad): elevated expression of CD10 in the tumor and stromal cells of both TCC and SCC and decreased expression of E-cad in the tumor cells of TCC are significantly associated with tumor progression, invasion, and metastasis (Omran, 2012).

Galectin-3: serum level of galectin-3 in patients with bladder cancer is shown to be significantly higher than controls. Higher expression of galectin-3 has been demonstrated in bladder cancer tissue than in normal bladder tissue. However, no noticeable correlation has been seen in serum galectin-3 concentration with the clinicopathological features such as stage and grade (Sakaki et al., 2008).

Conclusions

About half of patients suffering from MIBC die from their disease and present chemotherapy protocols do not increase their survival significantly. Although the survival of patients with NMIBC is good, tumor recurrence occur in about two-thirds of them (Kompier et al., 2010). Cancer researchers are currently investigating for bladder cancer biomarkers with the goal of decreasing the use of cystoscopy for diagnosis or surveillance of patients. Because of the close contact of urine with bladder tumors, the most investigated bladder cancer biomarkers are urinary markers. Many cancer markers have been proposed, but most of them could not enter market and clinic. The results of clinical trials for some of them have been promising and few have been approved for clinical use. Currently available data suggest that single biomarkers are inadequate for efficient patient surveillance (Frantzi et al., 2012). Therefore, different biomarkers combinations are being proposed for improvement of sensitivity and specificity of tests. In addition to combined tests reviewed in this article, a screening protocol has been recently suggested that could reduce unnecessary invasive follow-up testing. This sequential screening approach consists of home haematuria testing followed by NMP22 levels in urine, microsatellite analysis (MA), FGFR3 mutation analysis, and a custom methylation-specific test (Bangma et al., 2013). In addition to application of markers in cancer detection, they can be used for treatment decision. Novel therapies aiming at receptor tyrosine kinases or activated oncogenes have been promising in some cases. Therefore, mutations analysis of relevant oncogenes is necessary to find patients benefitting from these kinds of therapies (Kompier et al., 2010).

References

Abogunrin F, O'Kane HF, Ruddock MW, et al (2012). The impact of biomarkers in multivariate algorithms for bladder cancer diagnosis in patients with hematuria. *Cancer*, **118**, 2641-50.

Alvarez A, Lokeshwar VB (2007). Bladder cancer biomarkers: current developments and future implementation. *Curr Opin Urol*, **17**, 341-6.

Babjuk M, Kostirova M, Mudra K, et al (2002). Qualitative and quantitative detection of urinary human complement factor H-related protein (BTA stat and BTA TRAK) and fragments of cytokeratins 8, 18 (UBC rapid and UBC IRMA) as markers for transitional cell carcinoma of the bladder. *Eur Urol*, **41**, 34-9.

Bangma CH, Loeb S, Busstra M, et al (2013). Outcomes of a bladder cancer screening program using home hematuria testing and molecular markers. *Eur Urol*, **64**, 41-7.

Bergeron A, Picard V, LaRue H, et al (2009). High frequency of MAGE-A4 and MAGE-A9 expression in high-risk bladder cancer. *Int J Cancer*, **125**, 1365-71.

Bhagirath D, Abrol N, Khan R, et al (2012). Expression of CD147, BIGH3 and Stathmin and their potential role as diagnostic marker in patients with urothelial carcinoma of the bladder. *Clin Chim Acta*, **413**, 1641-6.

Brems-Eskildsen AS, Zieger K, Toldbod H, et al (2010). Prediction and diagnosis of bladder cancer recurrence based on urinary content of hTERT, SENP1, PPP1CA, and MCM5 transcripts. *BMC Cancer*, **10**, 646.

Chen CL, Lai YF, Tang P, et al (2012). Comparative and targeted proteomic analyses of urinary microparticles from bladder cancer and hernia patients. *J Proteome Res*, **11**, 5611-29.

Chen YT, Chen CL, Chen HW, et al (2010). Discovery of novel bladder cancer biomarkers by comparative urine proteomics using iTRAQ technology. *J Proteome Res*, **9**, 5803-15.

Cheung G, Sahai A, Billia M, Dasgupta P, Khan MS (2013). Recent advances in the diagnosis and treatment of bladder cancer. *BMC Med*, **11**, 13.

Chihara Y, Kanai Y, Fujimoto H, et al (2013). Diagnostic markers of urothelial cancer based on DNA methylation analysis. *BMC Cancer*, **13**, 275.

Dhawan D, Hamdy FC, Rehman I, et al (2006). Evidence for the early onset of aberrant promoter methylation in urothelial carcinoma. *J Pathol*, **209**, 336-43.

Eissa S, Kassim SK, Labib RA, et al (2005). Detection of bladder carcinoma by combined testing of urine for hyaluronidase and cytokeratin 20 RNAs. *Cancer*, **103**, 1356-62.

Feldman AS, Banyard J, Wu CL, McDougal WS, Zetter BR (2009). Cystatin B as a tissue and urinary biomarker of bladder cancer recurrence and disease progression. *Clin Cancer Res*, **15**, 1024-31.

Frantzi M, Makridakis M, Vlahou A (2012). Biomarkers for bladder cancer aggressiveness. *Curr Opin Urol*, **22**, 390-6.

Ghafouri-Fard S, Ghafouri-Fard S (2012). Immunotherapy in nonmelanoma skin cancer. *Immunotherapy*, **4**, 499-510.

Ghafouri-Fard S, Abdollahi DZ, Omrani M, Azizi F (2012). shRNA mediated RHOXF1 silencing influences expression of BCL2 but not CASP8 in MCF-7 and MDA-MB-231 cell lines. *Asian Pac J Cancer Prev*, **13**, 5865-9.

Ghafouri-Fard S, Modarressi MH (2009). Cancer-testis antigens: potential targets for cancer immunotherapy. *Arch Iran Med*, **12**, 395-404.

Ghafouri-Fard S, Modarressi MH (2012). Expression of cancer-testis genes in brain tumors: implications for cancer immunotherapy. *Immunotherapy*, **4**, 59-75.

Ghafouri-Fard S, Ousati Ashtiani Z, Sabah Golian B, Hasheminasab SM, Modarressi MH (2010). Expression of two testis-specific genes, SPATA19 and LEMD1, in prostate cancer. *Arch Med Res*, **41**, 195-200.

Gibanel R, Ribal MJ, Filella X, et al (2002). BTA TRAK urine test increases the efficacy of cytology in the diagnosis of low-grade transitional cell carcinoma of the bladder. *Anticancer Res*, **22**, 1157-60.

Goodison S, Rosser CJ, Urquidi V (2013). Bladder cancer detection and monitoring: assessment of urine- and blood-based marker tests. *Mol Diagn Ther*, **17**, 71-84.

Griffiths TR (2013). Current perspectives in bladder cancer management. *Int J Clin Pract*, **67**, 435-48.

Guo B, Luo C, Xun C, et al (2009). Quantitative detection of cytokeratin 20 mRNA in urine samples as diagnostic tools for bladder cancer by real-time PCR. *Exp Oncol*, **31**, 43-7.

Guo Y, Ying L, Tian Y, et al (2013). miR-144 downregulation increases bladder cancer cell proliferation by targeting EZH2 and regulating *Wnt signaling*. *FEBS J*, **280**, 4531-8.

Hanke M, Hoefig K, Merz H, et al (2010). A robust methodology to study urine microRNA as tumor marker: microRNA-126 and microRNA-182 are related to urinary bladder cancer. *Urol Oncol*, **28**, 655-61.

Ho CC, Tan WP, Pathmanathan R, Tan WK, Tan HM (2013). Fluorescence-in-situ-hybridization in the surveillance of urothelial cancers: can use of cystoscopy or ureteroscopy be deferred? *Asian Pac J Cancer Prev,* **14**, 4057-9.

Holterman DA, Diaz JI, Blackmore PF, et al (2006). Overexpression of alpha-defensin is associated with bladder cancer invasiveness. *Urol Oncol*, **24**, 97-108.

Hoque MO, Begum S, Topaloglu O, et al (2006). Quantitation of promoter methylation of multiple genes in urine DNA and bladder cancer detection. *J Natl Cancer Inst,* **98**, 996-1004.

Hu HY, Hu ZL, Wang HQ, Liu C, Yu SJ (2012). Nuclear matrix protein 22 and urinary cytology test in the diagnosis of bladder cancer: a meta-analysis. *Zhonghua Wai Ke Za Zhi*, **50**, 1126-30.

Irani J, Desgrandchamps F, Millet C, Toubert ME, et al (1999). BTA stat and BTA TRAK: A comparative evaluation of urine testing for the diagnosis of transitional cell carcinoma of the bladder. *Eur Urol*, **35**, 89-92.

Irmak S, Tilki D, Heukeshoven J, et al (2005). Stage-dependent increase of orosomucoid and zinc-alpha2-glycoprotein in urinary bladder cancer. *Proteomics,* **5**, 4296-304.

Johnen G, Gawrych K, Bontrup H, et al (2012). Performance of survivin mRNA as a biomarker for bladder cancer in the prospective study UroScreen. *PLoS One*, **7**, 16.

Karam JA, Lotan Y, Karakiewicz PI, (2007). Use of combined apoptosis biomarkers for prediction of bladder cancer recurrence and mortality after radical cystectomy. *Lancet Oncol*, **8**, 128-36.

Karbakhsh M, Dabbagh N, Shabani A, Tabibi A, Akhavizadegan H (2013). Age at diagnosis in bladder cancer: does opium addiction play a role? *Asian Pac J Cancer Prev*, **14**, 4723-5.

Kelly JD, Dudderidge TJ, Wollenschlaeger A (2012). Bladder cancer diagnosis and identification of clinically significant disease by combined urinary detection of Mcm5 and nuclear matrix protein 22. *PLoS One*, **7**, 40305.

Kohler CU, Bryk O, Meier S, et al (2013). Analyses in human urothelial cells identify methylation of miR-152, miR-200b and miR-10a genes as candidate bladder cancer biomarkers. *Biochem Biophys Res Commun*, **438**, 48-53.

Kompier LC, Lurkin I, van der Aa MN, et al (2010). FGFR3, HRAS, KRAS, NRAS and PIK3CA mutations in bladder cancer and their potential as biomarkers for surveillance and therapy. *PLoS One*, **5,** 13821.

Lai Y, Ye J, Chen J, et al (2010). UPK3A: a promising novel urinary marker for the detection of bladder cancer. *Urology*, **76**, 25.

Laurberg JR, Brems-Eskildsen AS, Nordentoft I, et al (2012). Expression of TIP60 (tat-interactive protein) and MRE11 (meiotic recombination 11 homolog) predict treatment-specific outcome of localised invasive bladder cancer. *BJU Int*, **110**, 9.

Lei Y, Yan S, Ming-De L, Na L, Rui-Fa H (2011). Prognostic significance of Aurora-A expression in human bladder cancer. *Acta Histochem*, **113**, 514-8.

Linden M, Segersten U, Runeson M, et al (2013). Tumour expression of bladder cancer-associated urinary proteins. *BJU Int*, **112**, 407-15.

Lindgren D, Liedberg F, Andersson A, et al (2006). Molecular characterization of early-stage bladder carcinomas by expression profiles, FGFR3 mutation status, and loss of 9q. *Oncogene*, **25**, 2685-96.

Lorenzi T, Lorenzi M, Altobelli E, et al (2013). HtrA1 in human urothelial bladder cancer: a secreted protein and a potential novel biomarker. *Int J Cancer*, **133**, 2650-61.

Matsuda R, Enokida H, Chiyomaru T, et al (2011). LY6K is a novel molecular target in bladder cancer on basis of integrate genome-wide profiling. *Br J Cancer*, **104**, 376-86.

Mengus C, Schultz-Thater E, Coulot J, et al (2013). MAGE-A10 cancer/testis antigen is highly expressed in high-grade non-muscle-invasive bladder carcinomas. *Int J Cancer*, **132**, 2459-63.

Miah S, Dudziec E, Drayton RM, et al (2012). An evaluation of urinary microRNA reveals a high sensitivity for bladder cancer. *Br J Cancer,* **107**, 123-8.

Myers-Irvin JM, Van Le TS, Getzenberg RH (2005). Mechanistic analysis of the role of BLCA-4 in bladder cancer pathobiology. *Cancer Res*, **65,** 7145-50.

Nana-Sinkam SP, Croce CM (2013). Clinical applications for microRNAs in cancer. *Clin Pharmacol Ther*, **93**, 98-104.

Nielsen ME, Gonzalgo ML, Schoenberg MP, Getzenberg RH (2006). Toward critical evaluation of the role(s) of molecular biomarkers in the management of bladder cancer. *World J Urol*, **24**, 499-508.

Nordentoft I, Birkenkamp-Demtroder K, Agerbæk M, et al (2012). miRNAs associated with chemo-sensitivity in cell lines and in advanced bladder cancer. *BMC Med Genomics*, **5**, 40.

Obara W, Ohsawa R, Kanehira M, et al (2012). Cancer peptide vaccine therapy developed from oncoantigens identified through genome-wide expression profile analysis for bladder cancer. *Jpn J Clin Oncol*, **42**, 591-600.

Omran OM (2012). CD10 and E-cad expression in urinary bladder urothelial and squamous cell carcinoma. *J Environ Pathol Toxicol Oncol*, **31**, 203-12.

Orenes-Pinero E, Corton M, Gonzalez-Peramato P, et al (2007). Searching urinary tumor markers for bladder cancer using a two-dimensional differential gel electrophoresis (2D-DIGE) approach. *J Proteome Res*, **6**, 4440-8.

Pesch B, Taeger D, Johnen G, et al (2013). Screening for bladder cancer with urinary tumor markers in chemical workers with exposure to aromatic amines. *Int Arch Occup Environ Health*, [Epub ahead of print].

Pignot G, Cizeron-Clairac G, Vacher S, et al (2013). microRNA expression profile in a large series of bladder tumors: identification of a 3-miRNA signature associated with aggressiveness of muscle-invasive bladder cancer. *Int J Cancer*, **132**, 2479-91.

Qin ZK, Yang JA, Ye YL, et al (2009). Expression of Bmi-1 is a prognostic marker in bladder cancer. *BMC Cancer*, **9**, 61.

Qin ZK, Yang JA, Zeng MS, et al (2008). Expression and clinical significance of Bmi-1 protein in bladder cancer. *Ai Zheng*, **27**, 1327-30.

Rahmani AH, Alzohairy M, Babiker AY, et al (2013). Implication of androgen receptor in urinary bladder cancer: a critical mini review. *Int J Mol Epidemiol Genet*, **4**, 150-5.

Rasmussen HH, Orntoft TF, Wolf H, Celis JE (1996). Towards a comprehensive database of proteins from the urine of patients with bladder cancer. *J Urol*, **155**, 2113-9.

Rosser CJ, Ross S, Chang M, et al (2013). Multiplex protein signature for the detection of bladder cancer in voided urine samples. *J Urol*, **190**, 2257-62.

Sakaki M, Oka N, Nakanishi R, et al (2008). Serum level of

galectin-3 in human bladder cancer. *J Med Invest*, **55**, 127-32.
Sanchini MA, Gunelli R, Nanni O, et al (2005). Relevance of urine telomerase in the diagnosis of bladder cancer. *JAMA*, **294**, 2052-6.
Sartini D, Muzzonigro G, Milanese G, et al (2013). Upregulation of tissue and urinary nicotinamide N-methyltransferase in bladder cancer: potential for the development of a urine-based diagnostic test. *Cell Biochem Biophys*, **65**, 473-83.
Scher MB, Elbaum MB, Mogilevkin Y, 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**, 2101-7.
Shariat SF, Savage C, Chromecki TF, et al (2011). Assessing the clinical benefit of nuclear matrix protein 22 in the surveillance of patients with nonmuscle-invasive bladder cancer and negative cytology: a decision-curve analysis. *Cancer*, **117**, 2892-7.
Sharma P, Gnjatic S, Jungbluth AA, et al (2003). Frequency of NY-ESO-1 and LAGE-1 expression in bladder cancer and evidence of a new NY-ESO-1 T-cell epitope in a patient with bladder cancer. *Cancer Immun*, **3**, 19.
Siegel R, Naishadham D, Jemal A (2013). Cancer statistics, 2013. *CA Cancer J Clin*, **63**, 11-30.
Smith ZL, Guzzo TJ (2013). Urinary markers for bladder cancer. *F1000Prime Rep*, **5**, 21.
Snowdon J, Boag S, Feilotter H, Izard J, Siemens DR (2012). A pilot study of urinary microRNA as a biomarker for urothelial cancer. *Can Urol Assoc J*, **15**, 1-5.
Srivastava AK, Singh PK, Srivastava K, et al (2013). Diagnostic role of survivin in urinary bladder cancer. *Asian Pac J Cancer Prev*, **14**, 81-5.
Stoeber K, Swinn R, Prevost AT, et al (2002). Diagnosis of genito-urinary tract cancer by detection of minichromosome maintenance 5 protein in urine sediments. *J Natl Cancer Inst*, **94**, 1071-9.
Sun YW, Xuan Q, Shu QA, et al (2013). Correlation of tumor relapse and elevated expression of survivin and vascular endothelial growth factor in superficial bladder transitional cell carcinoma. *Genet Mol Res*, **12**, 1045-53.
Svatek RS, Herman MP, Lotan Y, et al (2006). Soluble Fas-a promising novel urinary marker for the detection of recurrent superficial bladder cancer. *Cancer*, **106**, 1701-7.
Tabarestani S, Ghafouri-Fard S (2012). Cancer stem cells and response to therapy. *Asian Pac J Cancer Prev*, **13**, 5951-8.
Tauber S, Brunken C, Vierbuchen M (2011). Expression of the tumormarker p16INK4a in cytology specimens of the urinary bladder. A new means for early recognition and surveillance of urothelial cancer. *Urologe A*, **50**, 1130-3.
Tyan YC, Yang MH, Chen SC, et al (2011). Urinary protein profiling by liquid chromatography/tandem mass spectrometry: ADAM28 is overexpressed in bladder transitional cell carcinoma. *Rapid Commun Mass Spectrom*, **25**, 2851-62.
Urquidi V, Goodison S, Kim J, et al (2012a). Vascular endothelial growth factor, carbonic anhydrase 9, and angiogenin as urinary biomarkers for bladder cancer detection. *Urology*, **79**, 1181-6.
Urquidi V, Goodison S, Ross S, et al (2012b). Diagnostic potential of urinary alpha1-antitrypsin and apolipoprotein E in the detection of bladder cancer. *J Urol*, **188**, 2377-83.
Urquidi V, Kim J, Chang M, et al (2012c). CCL18 in a multiplex urine-based assay for the detection of bladder cancer. *PLoS One*, **7**, 37797.
Van Le TS, Myers J, Konety BR, Barder T, Getzenberg RH (2004). Functional characterization of the bladder cancer marker, BLCA-4. *Clin Cancer Res*, **10**, 1384-91.
Wang DL, Lan JH, Chen L, et al (2012). Integrin-linked kinase functions as a tumor promoter in bladder transitional cell carcinoma. *Asian Pac J Cancer Prev*, **13**, 2799-806.
Wang, XS, Zhang Z, Wang HC, et al (2006). Rapid identification of UCA1 as a very sensitive and specific unique marker for human bladder carcinoma. *Clin Cancer Res*, **12**, 4851-8.
Wszolek MF, Rieger-Christ KM, Kenney PA, et al (2011). A MicroRNA expression profile defining the invasive bladder tumor phenotype. *Urol Oncol*, **29**, 794-801.
Xiong D, Ye YL, Chen MK, et al (2012). Non-muscle myosin II is an independent predictor of overall survival for cystectomy candidates with early-stage bladder cancer. *Oncol Rep*, **28**, 1625-32.
Yang N, Feng S, Shedden K, et al (2011). Urinary glycoprotein biomarker discovery for bladder cancer detection using LC/MS-MS and label-free quantification. *Clin Cancer Res*, **17**, 3349-59.
Yin, B, Liu G, Wang XS, et al (2012). Expression profile of cancer-testis genes in transitional cell carcinoma of the bladder. *Urol Oncol*, **30**, 886-92.
Yoshino H, Seki N, Itesako T, et al (2013). Aberrant expression of microRNAs in bladder cancer. *Nat Rev Urol*, **10**, 396-404.
Yun SJ, Jeong P, Kim WT, et al (2012). Cell-free microRNAs in urine as diagnostic and prognostic biomarkers of bladder cancer. *Int J Oncol*, **41**, 1871-8.
Zheng Y, Rodrik V, Toschi A, et al (2006). Phospholipase D couples survival and migration signals in stress response of human cancer cells. *J Biol Chem*, **281**, 15862-8.