Molecular diagnosis of bladder cancer from urine

Daniel Tam Chuen-Chu[1], *Li Yuen-Yan*[1], *Kwok Ying-Kin*[1], *Chris Wai Kin-Lung*[1] *and Stephen Lo Tsui-Hoi*[2]

[1]Genepath Technology Limited
[2]Department of Pathology, Princess Margaret Hospital

Address for correspondence:
E-mail: cctam@netvigator.com

Introduction

Bladder cancer was the seventh most common cancer in Hong Kong in 2004.[1] Many of the tumor markers of bladder cancer can be found in urine. An ideal marker should be objective, highly sensitive and specific, noninvasive and easy to interpret. The current standard noninvasive bladder cancer marker - urine cytology, has relatively high specificity but with low sensitivity, and these can be as low as 73% and 37% respectively.[2] Therefore, a number of literatures reported different approaches to increase the sensitivity and specificity of the tests.

DNA based markers

Telomerase

Telomere has nucleotide sequences at the end of chromosomes that protect genetic stability during DNA replication and cell division. During each DNA replication cycle, about 200 nucleotides are lost. The telomere sequence is synthesized by a ribonucleoprotein with enzymatic activities called telomerase. Telomerase is an enzyme expressed by tumor cells that stabilized telomere length by adding hexameric repeats (TTAGGG) to the ends of the chromosome, and thus maintain the length of telomeres. Polymerase chain reaction (PCR)-based techniques, such as reverse transcriptase polymerase chain reaction (RT-PCR), and telomeric repeat amplification protocol (TRAP) assay have been developed to detect the presence of telomerase or its activities in cancer cell within urine.[3]

Regarding the RT-PCR assay, it measures the level of human telomerase reverse transcriptase (hTERT) messenger ribonucleic acid (mRNA).[4] hTERT is the catalytic subunit of telomerase. The sensitivity of hTERT RT-PCR assay and TRAP assay were reported to be between 70% and l00%, whereas the specificity were about 60% to 70%.[5]

The variations in sensitivity may be due to the low stability or telomerase and hTERT mRNA in urine. Besides, at least 50 cells expressing telomerase are required for a valid TRAP assay.[6] The inflammatory cells may increase the expression level of telomerase and result in false positive. Therefore, it may lower the specificity of the telomerase test. As a result, some researchers found telomerase activity to be nonspecific for malignancy. In summary, telomerase is a sensitive and accurate bladder tumor marker, provided the specimens are preserved to avoid loss of telomerase activity or hTERT mRNA degradation.

Oncogenes

The detection of mutated H-ras oncogene in urine can enhance the diagnostic accuracy of bladder cancer. When combining the result of urine cytology, it can enhance the accuracy of either test alone from 33% to 60%.[7] Besides, the loss of heterozygosity with consequent loss of expression of the retinoblastoma gene is well established in bladder cancer.[8] In addition, the detection of hypermethylation of tumor suppressor gene in urine sediment, such as p14(ARF), suggested bladder cancer.[9] However, most of the above tests are complex and only available in specialize laboratories.

Microsatellite analysis

Both voided urine and bladder wash can detect p53 mutation in cells which highly correlated with the actual tumor p53 status.[10] By analysing a number of microsatellite markers (repeating DNA sequences), errors in DNA replication resulting from faulty DNA repair mechanisms can be detected. In a study, 95% patients with bladder cancer could be detected through microsatellite analysis.[11] Repeated urinary microsatellite analysis using 20 different markers has been used to predict the risk of recurrent bladder tumors.[12] Of 11 patients who had recurrent tumors developed, urinary microsatellite analysis accurately detected 90% of the tumors, whereas urine cytology can only detect 13% of the patient who had a tumor recurrence. In general the sensitivity of microsatellite analysis has revealed sensitivity more than 90%.[13] Although the preliminary research finding is promising, further studies with larger sample size are required to confirm these early findings. Besides, the standardization of test procedure is important if the test is launched for routine use.

Cell based markers

ImmunoCyt/uCyt

The immunoCyt/uCyt (Diagnocure Inc., Quebec City, Quebec, Canada) is an immunofluorescence microscopy test that use monoclonal antibodies to identify the M344 (sialylated carbohydrate epitope on a mucin protein MAUB), 19A211 (cell surface glycoprotein), and the LDQ10 antigens on the surface of the voided cells. M344 expression has been reported in 70% of superficial bladder tumors.[14] The 19A211 is commonly found in low-grade tumors. Studies use ImmunoCyt showed a sensitivity of 70-80%. The specificity of the test ranges between 60-70%. When combined with cytology, the test may have 100% sensitivity to detect carcinoma in situ.[15] In summary, it is sensitive and reasonably specific for use in combination with voided urine cytology. However, a steep learning curve, constant quality control and observer-dependent inference can hinder the usage of this test.

Fluorescence in situ hybridization test

The Vysis UroVysion test (Abbott Laboratories, Abbott Park, IL, USA) is a multi-target, multi-color fluorescence in situ hybridization test that can detect cancer cells in bladder wash specimens. It detect the abnormal increase in the copy number of chromosome 3 (spectrum red), 7 (spectrum green), 17 (spectrum aqua), and homozygous deletions of the p16 locus 9p21 (spectrum gold).[2] This probe set was selected after examining a set of 10 different pericentromeric probes to the most frequently altered chromosomes in urothelial cancer in a detection set of 30 patients.

The cells are observed under a fluorescence microscope. The criteria for detecting bladder cancer are $\geqq 5$ cells with a gain of $\geqq 2$ chromosomes, $\geqq 10$ cells with a gain of 1 chromosome, or $\geqq 20\%$ of cells with a loss of 9p21 locus. The sensitivity is between 69-87%.[4] This test has good sensitivity to detect carcinoma in situ and high-grade tumor. However, the sensitivity is too low (36%-60%) in detecting low-grade tumors.

DD23

DD23 tumor marker (UroCor, Inc., Oklahoma City, OK, USA) is an immunohistochemical assay that visualizes tumor cells in urine specimens. It uses a monoclonal antibody that detects a protein dimer expressed on bladder cancer cells. In the studies, DD23 had high sensitivity, ranging between 70% and 80%.[16,17] It has relatively high sensitivity to detect both low grade (70%) and high grade (87%) tumors. However, the specificity is low (60%). Therefore, it should be used with urine cytology to improve the sensitivity of cytology in detecting low grade disease in particular.

Protein based markers

NMP22

NMP22 test (Matritech Inc., Newton, MA, USA) has two different forms. They are Enzyme-Linked ImmunoSorbent Assay (ELISA) test and the point-of-care device (NMP22 BladderChek test). NMP22 is a nuclear mitotic apparatus protein found in the nuclear matrix of all cell types. It is involved in DNA replication, RNA transcription and gene expression.[18] Patients with bladder cancer may have urinary NMP22 levels that are 25-fold higher than normal individuals.[19] Using 10U/ml as cut-off, the test can identify those patients who are likely to have local recurrence or those who have invasive diseases.

The NMP22 ELISA test utilized two monoclonal antibodies (mAb302-18 and mAb32-22). The sensitivity of this test varies from 48 to 100% and the specificity varies form 70 to 91%.[20] It has a high false-positive rate among patients with inflammatory conditions, renal or bladder calculi, foreign bodies, bowel interposition, other genitourinary cancers, and proteinuria.

The BladderChek test was evaluated in a prospective study involving 133 individuals who were at a higher risk for bladder cancer. The test had 55.7% sensitivity and 85.7% specificity in detecting bladder cancer.[21] As with the NMP22 ELISA, the sensitivity of the BladderChek test increased with tumor grade and stage. In another study, it was 4-fold more sensitive than cytology and was suggested for use in early detection of bladder cancer.[22] Besides, it is easy to administer and relatively inexpensive.

BTA test

Cellular proliferation in bladder cancer is associated with the presence of urinary tumoral antigens, which are not produced in normal cells. The original BTA test consist of a latex agglutination test carried out in several steps to detect basement membrane antigen complex in the urine. The BTA *stat* (Bard diagnostic sciences, Redmond, Wash., USA) is the second-generation qualitative point-of-care test which can detect a bladder tumor antigen, a high molecular weight protein related to the H factor of human complement. It is a one step immunochromatographic assay.[23] The BTA *stat* test is easy to perform and only required 5 drops of recently voided urine. The sensitivity of this test varies from 57 to 78% and the specificity varies 52 to 93%.[20]

The BTA TRAK test (Bard diagnostic sciences, Redmond, Wash., USA) is a quantitative enzyme immunoassay which requires two different monoclonal antibodies to bind the same antigen as the BTA *stat*. The extent of enzyme reaction is determined by measuring the absorbance at 405nm. The absorbance obtained is proportional to the concentration of antigen in the test sample in the range of 0-100U/ml and is compared with the absorbance obtained in controls. The TRAK normal range is defined as 0-14 U/ml.[24] The sensitivity of this test varies from 62 to 76% and the specificity varies from 51 to 98%.[20]

When comparing BTA assay, NMP22 test and urine cytology, both BTA and NMP22 showed higher sensitivity than voided urine cytology for low grade tumors, but this advantage was lost in the higher grade tumors. The specificity of cytology was higher than that of either test alone. For both the NMP22 and BTA tests, the high false-positive rates and relatively low positive predicative values can be improved by eliminating established factors that would interfere with test results. These factors include benign inflammatory conditions, renal and bladder calculi, current or prior foreign body in the urinary tract, interposition of bowel segment, other genitourinary neoplasms, and recent instrumentation.[25] Eliminating patients with these conditions can increase specificity of the BTA *stat* and NMP22 tests to values on par with urine cytology.

Hyaluronic acid and hyaluronidase

The hyaluronic acid (HA) is a non-sulfated glycosaminoglycan. It is a component of tissue matrix and fluids. HA maintains cartilage integrity, osmotic balance and homeostasis of water. It also interacts with specific cell surface receptors, and regulated cell adhesion, migration and proliferation. In bladder cancer tissue, the HA levels are 3 to 5 fold higher than that of normal tissue.[26] Hyaluronidase (HAase) is an endoglycosidase which produces degradation of HA. HAase levels are increased in tumor tissues which correlate with grading. In bladder cancer, it has been observed that HAase is 5 to 8 fold higher in grade 2 and grade 3 tumor tissue extract than in grade 1.[27]

The hyaluronic acid-hyaluronidase test is a combination of 2 ELISA-like assays that measure urinary hyaluronic acid and hyaluronidase levels. These levels are then normalized to urinary protein. The HA test detects bladder cancer regardless of tumor grade, whereas the HAase test detects grade 2 to grade 3 tumors. The combined hyaluronic acid-hyaluronidase test has had sensitivity between 83% and 94% to detect both primary and recurrent tumors.[4] The sensitivity to detect both low grade and high grade tumors is between 75% and 100%. A recent study consist of 340 individuals found that the HA test had 85.8% sensitivity and 61% specificity.[28] However, when using HYAL1 mRNA levels as detection target, the RT-PCR

had 90.8% sensitivity and 93.4% specificity to detect bladder cancer. The HyAL1 mRNA detection was higher in schistosomal bladder cancer (92%) than in non-schistosomal (77.3%) bladder cancer. The result was similar to the HA test as hyaluronidase mRNA detection in correlated with tumor grade. Further studies may need to carry out in order to validate this promising test.

Cytokeratins

The UBC-IRMA assay (IDL Biotech, Borlange, Sweden) and UBC-Rapid (IDL Biotech, Borlange, Sweden) test can detect cytokeratin 8 and 18 fragments in urine. UBC-IRMA is an ELISA test and UBC-rapid is a point-of-care test. In some study, UBC-IRMA test performed better than the BTA *stat* test and NMP22 test.[29,30] However, different investigators have used different cutoff values, which make the comparison of results difficult. The sensitivity of the tests varies from 35 to 79%. It depends on tumor grade and stage. The sensitivity is low when detecting low stage tumor.

The CYFRA21-1 test detects the soluble fragment of cytokeratin 19, the lowest molecular weight cyokeratin expressed by bladder tumor cells and normal urothelium. Studies showed a sensitivity of 96% and specificity of 74%.[31] However, this test has a high rate of false positive results in patients treated with BCG. In summary, cytokeratin markers have reasonable sensitivity but have low specificity among patients with benign inflammatory conditions. It may limit the clinical applicability.

Survivin

Survivin is an inhibitor of apoptosis. It has been reported that it can be detected in the urine of all bladder cancer patient.[32] Subsequent studies have confirmed the high sensitivity and specificity of survivin.[33] It had the advantage over NMP22 and urine cytology in detecting grade 1 tumors and carcinoma in situ, and it may also help to determine response to intravesical therapy by being higher in non-responders. However, large scale studies are needed in order to establish the cutoff value and allow a better clinical judgment on this new marker.

New molecular markers discovery by gene expression profiling

In the past, researchers studied a few genes at a time. We can now study a large number of genes in tumor using high throughput gene expression profiling.[34] In order to identify novel prognostic markers in tumor, microarrays are used to detect the presence of mRNAs that have been transcribed from genes for protein production. First, RNA is extracted from cells, and then converts to complimentary DNA (cDNA). The copies may be amplified by RT-PCR. Fluorescent tags are incorporated into the newly synthesized cDNA. A cDNA molecule that contains a sequence complementary to one of the single stranded probe sequences will hybridize to a spot at which the complementary probes are affixed. A microarray may contain a sequence complementary several thousands of these spots or an entire genome. By using the microarray scanner, the spot will fluoresce and record by computer. cDNA amplified from a tumor sample and normal control will fluoresce differently on the same spot. Increased or decreased fluorescence intensity indicates that cells in the sample have recently transcribed a gene that contains the probed sequence. The intensity of the fluorescence is roughly proportional to the number of copies of a particular mRNA that were present and thus roughly indicates the activity or expression level of that gene. As microarrays contain a large number of genes target that can be analyzed, a genetic profile of which genes in the genome are active or inactive can be obtained.

Differential expression of specific genes identified as molecular signatures is then confirmed by quantitative RT-PCR and /or immunohistochemistry. By using this technology, we are able to identify novel prognostic markers in different cancers. For example, Dyrskjot and associates performed full genome analysis to identify a 45 gene signature that signals disease progression and clinical outcome.[34] They are involved in cell differentiation and regulation of apoptosis. Using this genetic profile, the researcher could predict disease progression in an independent set of bladder tumor tissues in a statistically significant manner.

Proteomics profiling as a way to discovery urinary biomarkers

The advent of 2-D electrophoresis and protein mass spectrometry has enabled a new approach to biomarker discovery. It can be done by large scale profiling of the protein complement of urine. Large scale proteomics profiling of normal human urine samples has revealed the presence of at least 1000 different protein gene products and many more peptide fragments of larger proteins.[35] The goal of proteomics profiling in urine is to discovery of urinary protein excretion profiles that can be used clinically for tasks such as early detection of disease, classification of disease, assessment of prognosis, choice of therapeutic agents and monitoring of a particular therapeutic regimen.

The basic workflow for the development of clinically applicable protein biomarker assays are discovery of presumptive biomarkers form well-defined patient populations, validation of these biomarkers with regard to their abilities to make useful predictions in patient populations, and implementation, which involved the development of clinical assay and regulatory approval, etc.[35]

Current 2-D electrophoresis methods used to identify serum and urine markers involved isoelectric focusing, followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[36] Advantages of this technology include the ability to detect both high and low-molecular mass proteins, and easier identification of protein spots by mass spectrometry. The disadvantages of this technique include the need for technical skill, moderate reproducibility, labor intensive and time consuming. One of examples of bladder cancer marker discovered though 2-D electrophoresis was the nuclear matrix proteinase. It was then developed into ELISA test.

Mass spectrometry is a powerful technique to identify biomarkers from urine and plasma. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF-MS) is the method that compares the protein profiles in the urine of normal patients and those with bladder cancer to identify unique biomarkers.[36] The data obtained is then coupled to database searching analyzed by tandem mass spectrometry analysis which allows a high level of certainty to identify proteins based on molecular mass as well as peak pattern in order to obtain more information of the peptide sequences.

In summary, the large-scale applicability of proteomic approaches for diagnosing bladder cancer may not be feasible at the present time. However, those techniques are essential to identify potential biomarkers. More conventional test such as ELISA, RT-PCR, can be developed for routine clinical uses.

New bladder tumor markers and prognostic markers

There were a number of new tumor markers and prognostic markers proposed every year. In the past years, new tumor markers identified were expression pattern of BLCAI,[37] free NDA in urine,[38] level of ADAM12 mRNA,[39] serum and urine clusterin,[40] urine prothymosin-alpha,[41] and expression of cytokeratin 20 in urine cytology smears.[42] Besides, the following novel prognostic markers were being identified, they were thymidine phosphorylase expression,[43] expression of E-cadherin and alpha-, beta-gamma-catenins,[44] elevated urinary levels of soluble Fas,[45] hydronephrosis[46] and gene expression of ERCC1,[47]. Although significant progress of markers identification were made every year, further studies and larger numbers are needed to define the above markers as a reliable indicator of bladder cancer.

Conclusion

Urine is one of the most studied samples for bladder tumor markers. Most of them have higher sensitivity but lower specificity than cytology. Several promising new markers are under evaluation. The availability of modern genomic and proteomic technologies provides us a high throughput and highly accurate platform to screen out novel tumor markers for clinical diagnosis. Ultimately, these excellent potential markers will no doubt become available in the future and be applied in the clinical arena.

References

1. Hospital Authority, Hong Kong Cancer Registry, 2007. Available from URL:http://www3.ha.org.hk/cancereg/.
2. Sarosdy MF, Schellhammer P, Bokinsky G et al. Clinical evaluation of a multi-target fluorescent in situ hybridization assay for detection of bladder cancer. J Urol 2002; 168:1950-4.
3. Holt SE, Shay JW, Wright WE. Refining the telomere-telomerase hypothesis of aging and cancer. Nat Biotechnol 1996; 14:836-9.
4. Lokeshwar VB, Habuchi T, Grossman HB et al. Bladder tumor markers beyond cytology: International Consensus Panel on bladder tumor markers. Urology 2005; 66:35-63.
5. Veltri RW and Makarov DV. Nucleic acid-based marker approaches to urologic cancers. Urol Oncol 2006; 24:510-27.
6. Kavaler E, Landman J, Chang Y et al. Detecting human bladder carcinoma cells in voided urine samples by assaying for the presence of telomerase activity. Cancer 1998; 82:708-14.
7. Fitzgerald JM, Ramchurren N, Rieger K et al. Identification of H-ras mutations in urine sediments complements cytology in the detection of bladder tumors. J Natl Cancer Inst 1995; 87:129-33.
8. Xu HJ, Cairns P, Hu SX et al. Loss of RB protein expression in primary bladder cancer correlates will loss of heterozygosity at the RB locus and tumor progression. Int J Cancer 1993; 53:781-4.
9. Dulaimi E, Uzzo RG, Greenberg RE et al. Detection of bladder cancer in urine by a tumor suppressor gene hypermethylation panel. Clin Cancer Res 2004; 10: 1887-93.
10. Prescott JL, Montie J, Pugh TW et al. Clinical sensitivity of p53 mutation detection in matched bladder tumor, bladder wash, and voided urine specimens. Cancer 2001; 91:2127-35.
11. Mao L, Schoenberg MP, Scicchitano M et al. Molecular detection of primary bladder cancer by microsatellite analysis. Science 1996; 271:659-62.
12. Steiner G, Schoenberg MP, Linn JF et al. Detection of bladder cancer recurrence by microsatellite analysis of urine. Nat Med 1997; 3:621-4.
13. Goessl C, Muller M, Straub B et al. DNA alteration in body fluids as molecular tumor markers for urological malignancies. Eur Urol 2002; 41:621-4.
14. Messing EM, Teot L, Korman H et al. Performance of urine test in patients monitored for recurrence of bladder cancer: a multicenter study in the United States. J Urol 2005; 174:1238-41.
15. Mian C, Lodde M, Comploj E et al. The value of the ImmunoCyt/uCyt+ test in the detection and follow-up of carcinoma in situ of the urinary bladder. Anticancer Res 2005; 25:3641-4.
16. Gilbert SM, Veltri RW, Sawczuk A et al. Evaluation of DD23 as a marker for detection of recurrent transitional cell carcinoma of the bladder in patients with a history of bladder cancer. Urology 2003; 61:539-43.
17. Sawczuk IS, Pickens CL, Vasa UR et al. DD23 Biomarker: a prospective clinical assessment in routine urinary cytology specimens from patients being monitored for TCC. Urol Oncol 2002; 7:185-90.
18. Bidwell JP, Fey EG, van Wijnen Aj et al. Nuclear matrix proteins distinguish normal diploid osteoblasts from osteosarcoma cells. Cancer Res 1994; 54:28-32.
19. Keesee SK, Briggman JV, Thill G et al. Utilization of nuclear matrix proteins for cancer diagnosis. Crit Rev Eukaryot Gene Expr 1996; 6:189-214.
20. Bassi P, De Marco V, De Lisa A et al. Non-invasive diagnostic tests for bladder cancer: a review of the literature. Urol Int 2005; 75:193-200.
21. Grossman HB, Messing E, Soloway M et al. Detection of bladder cancer using a point of-care proteomic assay. JAMA 2005; 293:810-6.
22. Tomera KM,. NMP22 BladderChek Test: point-of-care technology with life- and money-saving potential. Expert Rev Mol Diagn 2004; 4:783-94.
23. Waksg IK, Keane PF, Ishak LM et al. The BTA stat test: a tumor marker for the detection of upper tract transitional cell carcinoma. Urology 2001; 58:532-5.
24. Schamhart DH, de Reijke TM, van der Poel HG et al. The bard BTA test: its mode of action, sensitivity and specificity, compared to cytology of voided urine, in the diagnosis of superficial bladder cancer. Eur Urol 1998; 34:99-106.
25. Sharma S, Zippe CD, Pandrangi L et al. Exclusion criteria enhance the specificity and positive predictive value of NMP22 and BTA stat. J Urol 1999; 162:53-7.

26. Lokeshwar VB, Obek C, Soloway MS et al. Tumor-associated hyaluronic acid: a new sensitive and specific urine marker for bladder cancer. Cancer Res 1997; 57:773-7
27. Pham HT, Block NL, Lokeshwar VB. Tumor-derived hyaluronidase: a diagnostic urine marker for high-grade bladder cancer. Cancer Res 1997; 57:778-83.
28. Eissa S, Kassim SK, Labib RA et al. Detection of bladder carcinoma by combined testing of urine for hyaluronidase and cytokeratin 20 RNAs. Cancer 2005; 103:1356-62.
29. Mian C, Lodde M, Haitel A et al. Comparison of the monoclonal UBC-ELISA test and the NMP22 ELISA test for the detection of urothelial cell carcinoma of the bladder. Urology 2000; 55:223-6.
30. Mian C, Lodde M, Haitel A et al. Comparison of two qualitative assays, the UBC rapid test and the BTA stat test, in the diagnosis of urothelial cell carcinoma of bladder. Urology 2000; 56:228-31.
31. Pariente JL, Bordenave L, Michel P et al. Initial evaluation of CYFRA 21-1 diagnostic performances as a urinary marker in bladder transitional cell carcinoma. J Urol 1997; 158:338-41.
32. Smith SD, Wheeler MA, Plescia J et al. Urine detection of surviving and diagnosis of bladder cancer. JAMA 2001; 285:324-8.
33. Shariat SF, Casella R, Khoddami SM et al. Urine detection of surviving is a sensitive marker for the noninvasive diagnosis of bladder cancer. J Urol 2004; 171:626-30.
34. Liang P, Pardee AB. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 1992; 257:967-71.
35. Pisitkum T, Johnstone R, Knepper MA. Discovery of urinary biomarkers. Mol Cell Proteomics 2006; 5:1760-71.
36. Lee SW, Lee KI, Kim JY. Revealing urologic diseases by proteomic techniques. J Chromatogr B Analyt Technol Biomed Life Sci 2005; 815:203-13.
37. Myers-Irvin JM, Landsittel D, Getzenberg RH. Use of the novel marker BLCA-1 for the detection of bladder cancer. J Urol 2005; 174:64-8.
38. Zancan M, Facnceschini R, Mimmo C et al. Free DNA in urine: a new marker for bladder cancer? Preliminary data. Int J Biol Markers 2005; 20:134-6.
39. Frohlich C, Albrechtsen R, Dyrskjot L et al. Molecular profiling of ADAM12 in human bladder cancer. Clin Cancer Res 2006; 12:7359-68.
40. Stejskal D, Fiala RR. Evaluation of serum and urine clusterin as a potential tumor marker for urinary bladder cancer. Neoplasma 2006; 53:343-6.
41. Tzai TS, Tsai YS, Shiau AL et al. Urine prothymosin-alpha as novel tumor marker for detection and follow-up of bladder cancer. Urology 2006; 67:294-9.
42. Bhatia A, Dey P, Kumar Y et al. Expression of cytokeratin 20 in urine cytology smears: a potential marker for the detection of urothelial carcinoma. Cytopathology 2007; 18:84-6.
43. Aoki S, Yamada Y, Nakamura K et al. Thymidine phosphorylase expression as a prognostic marker for predicting recurrence in primary superficial bladder cancer. Oncol Rep 2006; 16:279-84.
44. Ckauritte A, Lascombe I, Fauconnet S et al. Expression of E-cadherin and alpha-, beta-, gamma-catenins in patients with bladder cancer: identification of gamma-catenin as a new prognostic marker of neoplastic progression in T1 superficial urothelial tumors. Am J Clin Pathol 2006; 125:119-26.
45. Svatek RS, Herman MP, Lotan Y et al. Soluble Fas-a promising novel urinary marker for the detection of recurrent superficial bladder cancer. Cancer 2006; 106:1701-7.
46. Bartsch GC, Kuefer R, Gshwend JE et al. Hydronephrosis as a prognostic marker in bladder cancer in a systectomy-only series. Eur Urol 2007; 51:690-7; discussion 697-8.
47. Bellmunt J, Paz-Ares L, Cuello M et al. Gene expression of ERCC1 as a novel prognostic marker in advanced bladder cancer patients receiving cisplatin-based chemotherapy. Ann Oncol 2007; 18:522-8.