Telomerase and Other Novel Approaches to Bladder Cancer Detection

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ABBREVIATIONS: hTERT = human telomerase reverse transcriptase; hTR or hTERC = human telomerase RNA component; PCR = polymerase chain reaction; RUO = research use only; TRAP = telomeric repeat amplification protocol.

INDEX TERMS: apoptosis; bladder cancer; cystoscopy; cytology; microsatellite; polymerase chain reaction; senescence; telomerase.

Clin Lab Sci 2008;21(3):185

LEARNING OBJECTIVES

1. List laboratory methods for bladder cancer diagnosis.
2. Describe the function of telomerase in chromosomal replication.
3. Describe the pathologic action of telomerase in tumors.
4. Describe laboratory methods for monitoring telomerase activity.

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Over 68,000 new cases of urinary bladder cancer are expected in the United States in 2008. Of these, an estimated 13,000 people will die of this disease. Urinary bladder cancer is the fourth most common new cancer in men and the ninth most common in women, with incidence rates of 30.0 and 7.1 per 100,000, respectively.[1]

Bladder cancer can be divided into two broad categories relating to the severity of disease. The first affects 70% of the patients and is characterized by low-grade tumors with frequent subsequent recurrence. Patients with low-grade malignancies have a good prognosis and low mortality rate. The remaining 30% of patients have high-grade tumor lesions. While both categories are associated with frequent recurrences, patients with high-grade lesions are at high risk for metastasis to other organs and tissues. Patients in this category have particular need for accurate diagnosis and staging in order to improve survival rates.[2]

The greatest utility for a bladder cancer-screening program is in high-risk populations, i.e., those who have previously had urological cancer. The relatively low incidence rate (44 patients per 100,000 population) makes a general screening program unlikely due to the risks and costs associated with cystoscopy and cytology, the current screening methods.[3] However, screening individuals at high risk is beneficial for early bladder cancer detection. Thus, there is a real need for an accurate and non-invasive screening assay with high sensitivity and specificity to be used in these populations.

Urine tumor markers are a promising area of oncologic medicine with the potential for assays that are non-invasive, cost-efficient, and more sensitive than traditional methodologies. Potential applications for urine tumor markers are fourfold: recurrent testing to detect recurring disease, an adjunct with cytology to increase sensitivity and specificity, a more accurate and less costly substitute for urine cytology, and as a directing mechanism for cystoscopy follow-up.[4] Current FDA approved assays available for bladder cancer detection are BTA stat®, BTA TRAK®, NMP22®, and Immunocyst®. However, the available assays have limitations that restrict the generalizability of the reported results. New methods must be studied in a quest for an assay that is more sensitive,

specific, easy to perform, reproducible, and cost-efficient. There are several bladder tumor markers in early stages of development, not yet FDA approved. A new methodology that holds promise for these characteristics is the detection of telomerase in the urine. The potential relevance of telomerase for diagnosing bladder cancer comes from two main findings. Telomerase is an enzyme present in highly proliferative healthy and cancerous tissue, specifically bladder transitional cell carcinoma. In addition, the sensitivity of the telomeric repeat amplification protocol allows the detection of this enzyme in exfoliated cells collected in normally voided urine or in bladder washings collected during cystoscopy.[5] Additionally, six other commercially available urine-based bladder markers tests will be reviewed: BLCA-4, hyaluronic acid/hyaluronidase, Lewis X antigen, microsatellite markers, Quanticyst®, and Survivin.

CURRENT DIAGNOSTIC ASSAYS FOR BLADDER CANCER

FDA-approved bladder tumor markers

BTA Stat®. The BTA stat test (Polymedco; Cortlandt Manor NY) is a point-of-care testing device using monoclonal antibodies to detect the presence of complement factor H or related proteins from a urological malignancy. Studies involving BTA stat report high sensitivity for both low and high-grade tumors. The sensitivity for low-grade lesions is 50%-89%, and high-grade lesions have 89% sensitivity. Clinical specificity is 60%-92%.[6] The manufacturer reports no interference from urinary compounds and sediment such as red blood cells, white blood cells, or proteins. However, false positives have been reported in the literature whenever there is blood in the urine, because plasma contains complement factor H that can react with the antibody in the test.[7] This could occur in common events such as trauma, urinary tract infections, and renal calculi, and makes this assay unable to differentiate these conditions from urological cancer. The specificity of the BTA stat test is reduced to 20% in patients who have received intravesical immunotherapy or chemotherapy within the last three months, further limiting its utility in patients with a history of bladder cancer.[8]

BTA TRAK®. The BTA TRAK test (Polymedco; Cortlandt Manor NY) is in the same product family as the BTA Stat and is a quantitative test using a sandwich immunoassay to measure levels of human complement factor H-related protein. This test requires trained personnel and a reference laboratory. The BTA TRAK reports a sensitivity of 62%-77% for low-grade lesions, and 89%-92% for high-grade tumors.[9-12] This test has not met widespread acceptance due to a specificity of 48%-70%, indicating a high false-positive rate. As with the BTA stat test, benign genitourinary conditions, particularly hematuria, yield false positive results.[9,10]

NMP22®. The Nuclear Matrix Protein 22 test (Matritech; Newton MA) targets mitotic protein fragments expressed in bladder cancer. Nuclear matrix proteins make up the framework of a cell's nucleus and play a role in gene expression. NMP22 localizes with the spindle poles during mitosis and regulates chromatid and daughter cell separation.[13] This protein is present in bladder cancer cells in significantly higher amounts that in normal cells. The NMP22 assay is a quantitative enzyme immunoassay using two monoclonal antibodies to detect the protein. Specimens are tested on a lateral flow immunochromatographic strip with two separate antibodies, one capture and one reporter. The NMP22 test has a highly variable reported sensitivity and specificity. A recent study by Miyanaga and others compared the urine NMP22 test with voided urine cytology in high-risk patients. The NMP22 test had a sensitivity of 55.7% and a specificity of 85.7% with a false-positive rate of 35%.[14] The high false positive rate is problematic for screening purposes, due to the invasive nature of the follow-up testing. Patients must be excluded from the use of this assay based on six criteria: benign inflammatory or infectious conditions, renal or bladder calculi, foreign body (stent or nephrostomy tube), bowel interposition, other genitourinary cancers, and instrumentation.[15] Other problems with this assay include the instability of the protein in voided urine (specimen must be collected into a vial containing preservative and tested or frozen within 48 hours), and protein interferences from red and white blood cells.[16]

Immunocyst®. The Immunocyst assay (Diagno-Cure Inc.; Sainte-Foy Quebec, Canada) is a test combining cytology and immunofluorescence. The assay uses three fluorescent monoclonal antibodies to tag cancerous cells exfoliated in urine sediment. The markers of interest are carcinoembryonic antigen and mucins found on malignant cells. Tagged cells are visualized using immunofluorescent microscopy.[17] This test requires at least 40 mL of urine that is fixed immediately with 50% ethanol. It must be performed in a reference laboratory with personnel trained in fluorescent microscopy. ImmunoCyst has been shown to have a sensitivity of 50%-100%. Specificity has been reported as 69%-79%.[18-20] Most of the studies in the literature evaluated patients with a history of bladder cancer. Generalizability may be limited in more heterogeneous groups. Deficiencies of this methodology include high inter-observer variability and the dependence of this assay on intact exfoliated cells.

RESEARCH USE ONLY ASSAYS

Telomeric repeat amplification protocol assay

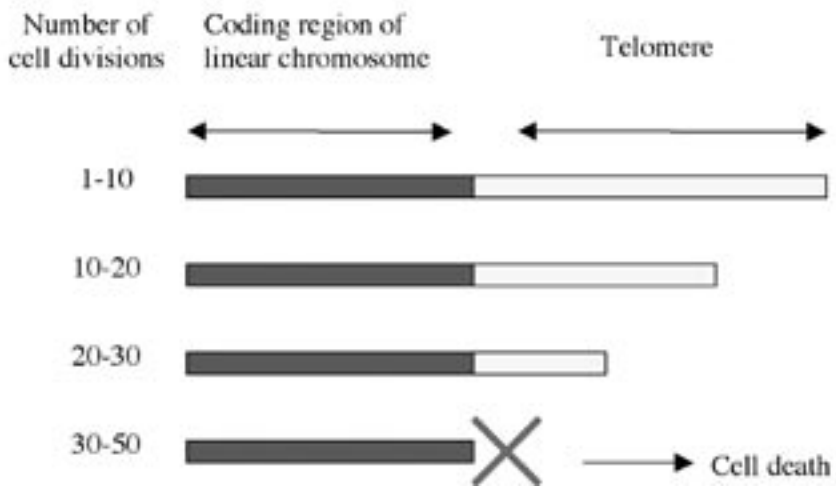
Historical background. Human cells contain 46 chromosomes that must be replicated in order to divide and confer life. At the end of each chromosome is an area of repeated base pair sequences, thousands of nucleotides, in the pattern TTAGGG. This area, a telomere, is a buffer that serves to protect the chromosome from loss of genetic material during replication. One strand of the 2-stranded linear chromosome is called the leading strand and other, the lagging strand. The leading strand is replicated linearly in a continuous manner, while the lagging strand is replicated in sections of discrete fragments (Okazaki fragments).[21] The replication is accomplished by adding multiple RNA primers and adding base pairs "backward" along the chromosome. At the very end of the telomere, there will inevitably be a section of DNA that is too short to add the appropriate primer and so this section of DNA will not be replicated. Over time, a considerable amount of DNA material is lost in replication, as approximately 50-100 bp are lost during each cell division. This is called the "end replication problem" (Figure 1). A current theory of cell aging is based on this concept. Eventually, the DNA will be vulnerable to end degradation and end-to-end fusions with other chromosomes as it is left unprotected by the shortened telomere region. Some view this mechanism as a natural "time clock" of the cell, allowing a finite number of cellular replications, also called the Hayflick limit of a cell. This barrier to infinite replication provides a cancer block, inhibiting uncontrolled growth. Aging of the cell and the programmed death that follows (apoptosis) is called cellular senescence and is normal for all somatic cells.[22]

Telomerase is an enzyme composed of both RNA and proteins (a ribonucleoprotein enzyme complex) that uses reverse transcriptase to synthesize DNA material. Telomerase actually maintains telomere length at the end of each chromosome in the human genome. This lengthening infers a type of "immortality" as the genetic material of a cell never ages or senesces. Telomerase is normally present in cells that must proliferate quickly and frequently. These cells include those in fetal tissue, male germ line tissues, and other renewable cells such as hematopoietic stem cells, basal cells of the epidermis, proliferative endometrial cells, and intestinal crypt cells. Other somatic cells do not exhibit telomerase activity and are subject to cellular senescence.[23]

Different cell lineages in the body maintain differing levels of telomerase activity. Stem cells, such as those in the bone marrow, contain low levels of telomerase activity while they are not proliferating. Upon becoming proliferative, they express greatly increased levels of telomerase. However, these cells clearly do not live forever and therefore must lose telomere length despite high levels of telomerase activity during certain cycles in their lifespan.[24]

Cancer cells do not lose telomere length and are referred to as "immortal".[23] The two major components of telomerase are the protein component, a catalytic subunit named hTERT (human telomerase reverse transcriptase), and the RNA component named hTR or hTERC (human telomerase RNA component). Shay and Wright demonstrated that introduction of the hTERT components into cells in culture results in increased telomere length and stabilization of the cellular genome.[25] It is logical to assume that inhibition of this gene would cause reduction of telomerase activity and might provide a basis for cancer therapeutics (Figure 2).[26]

Figure 1. Telomere length and cell death

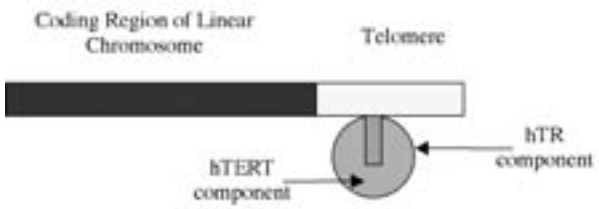


With each cell replication, the linear chromosome becomes shortened. At a finite number of cell divisions, telomere length becomes critical, leading to cell degradation and death.

Telomerase, present in large amounts in neoplastic tissue, is exploited by cancer in order to proliferate rapidly and be unchecked by replicative senescence. Approximately 85%-90% of all human tumors and tumor-derived cell lines demonstrate telomerase, shown by the telomeric repeat amplification protocol (TRAP) assay.[23] It is important to note, however, that the tissues adjacent to the tumor do not exhibit telomerase activities. Due to the almost complete correlation between the presence of malignancy and the reactivation of telomerase, this enzyme is particularly appropriate for diagnostic modalities to detect cancer. The literature reports a clinical sensitivity range of 70%-86% and a specificity range of 60%-90% for all TRAP methods combined.[27]

Originally, radioactive isotopes were used in functional reactions to identify telomerase products.[28] This, however useful, was not a plausible option for widespread clinical use for the detection of telomerase due to concerns about radioactivity hazard.[29] A new approach for telomerase detection called the telomeric repeat amplification protocol, or TRAP assay, was introduced in 1994 by Kim and others.[30] In this method, sample telomerase extends the substrate oligonucleotide by adding telomeric repeats to the reaction. This method uses a substrate primer to specifically amplify telomerase products via polymerase chain reaction (PCR) and is preferable because it does not depend on radioactive endpoints. PCR technology allows small amounts of telomerase to be detected in minimum volumes with increased sensitivity, speed, and efficency. All current TRAP assays are modifications based on this original PCR concept, and all are currently available for research use only (RUO). The literature reports a clinical sensitivity range of 70%-86% and a specificity range of 60%-90% for all TRAP methods combined. Limitations of the TRAP assay are sample management and analytical limitations.[31] Telomerase is a heat sensitive enzyme. Urine must be tested within 24 hours, or pelleted and stored at -85°C to -75°C or kept on dry ice. Telomerase in frozen cells or tissues is stable for at least one year at -85°C to -75°C.[5] The test limitations are PCR amplification artifacts such as primer-dimer artifact due to suboptimal PCR conditions, PCR carryover contamination, and RNAse contamination. Since PCR amplifies very small amounts of DNA to detectable levels, special care must be taken to ensure that cross-contamination does not occur. Sources of PCR product contamination are the gel box and buffer, contaminated pipettes and tips, tube racks, notebooks, lab coats, and other items exposed to amplified PCR products. Sources of RNase contamination are solutions and tubes not treated with RNase inhibitor, and any equipment handled without gloves. These contamination issues are critical and must be minimized to maintain test quality.

Figure 2. Schematic representation of telomerase molecule and its association with the linear chromosome



Other assays under investigation

BCLA-4. BCLA-4 is a nuclear transcription factor present in bladder tumors and adjacent benign areas of the bladder, but not in benign urothelium. BCLA-4 is one of six such factors which are promising tumor markers in bladder cancer detection. This protein is tested by an ELISA on voided urine. Preliminary studies indicate a sensitivity of 89%-96% with a specificity of 100% for bladder cancer.[32,33] Further studies with randomized multi-center trials are needed to confirm the usefulness of BCLA-4.

Hyaluronic acid, hyaluronidase. Hyaluronic acid is a non-sulfated glycosaminoglycan found normally in tissue and body fluids. Furthermore, it has been found in large amounts in cancerous tissue (2.5 to 6.5-fold in bladder cancer patients). In tumors, it supports metastasis and blocks immune surveillance.[34] The test to detect hyaluronic acid is based on competitive binding in which hyaluronic acid present in the urine specimen competes with hyaluronic acid coated on microtiter wells. Hyaluronidase is an endoglycosidase that degrades hyaluronic acid into smaller fragments. These fragments promote the formation of blood vessels around a tumor. Hyaluronidase in cancerous tissue is increased 3.0-7.0 fold compared to normal tissue. The hyaluronidase assay uses hyaluronic acid present in the urine specimen to degrade hyaluronic acid bound to microtiter wells. In each assay, hyaluronic acid binding protein is used to detect the product of interest. Both tests use color detection and internal standards to determine the amounts of hyaluronic acid or hyaluronidase in the sample. To date, only six studies have examined this methodology for bladder cancer detection, resulting in an overall sensitivity of 89%-91% and a specificity of 73-84%.[35,36] These tests are promising tumor markers with initial ideal characteristics. However, more studies are needed to validate its sensitivity and specificity as well as to define its limitations.

Lewis X antigen. Lewis related blood group antigens are cell surface molecules with four subclasses. Only the Lewis X group antigens are associated with bladder cancer.[37] This antigen is present only on tumor cells of transitional cell carcinoma and does not correlate with tumor stage or grade. Lewis X antigens are detected using immunostaining on formalin fixed bladder tissue. Several studies have been performed and reported a sensitivity of 80%-89% and a specificity of 49%-89 %.[38,39] Higher sensitivity can be achieved when two consecutive voided urine samples are tested. The testing for Lewis X antigens has shown promise, but further testing on heterogeneous patient populations is needed to confirm the preliminary data.

Microsatellite markers. Microsatellites are sections of DNA containing highly polymorphic repeats of 1-4 base pairs. Mutations in these areas are markers of neoplasia. In bladder cancer, chromosomes 4p, 8p, 9p, 11p, and 17p often display loss of heterogeneity in these microsatellite sections. These markers can be detected in voided urine by extracting and analyzing the DNA using PCR. Several studies have been conducted using a panel of microsatellite markers with promising results. Overall sensitivity was 72%-92% and specificity, 80%-100%.[40,41] These studies mainly involved small numbers of patients and large multi-center trials are needed to confirm sensitivity and specificity. Disadvantages of this method are a need for expensive equipment and trained personnel.

Quanticyst®. Quanticyst is an automated system that uses karyometric image analysis to evaluate nuclear shape and DNA content of exfoliated bladder cells obtained through catheterized bladder washing. The test involves fixing and staining exfoliated cells and importing the images to a computerized image analysis system attached to a microscope. It uses information obtained from the software to assign a risk score for bladder cancer as low, intermediate, or high. Quanticyst is not currently commercially available due to manufacturing difficulties. Overall sensitivity for the Quanticyst system has been suggested to be 45%-69% for the detection of bladder cancer, with a specificity of 70%-93%.[42-44] Quanticyst has demonstrated higher sensitivity than cytology in detection of low-grade tumors. However its sensitivity in high-grade tumors is less than that of cytology. Patients involved in this testing must undergo a catheterized specimen collection, which defeats the non-invasive appeal of most bladder tumor marker tests in urine. Additional disadvantages of the Quanticyst are the requirement of highly complex technical expertise and expensive equipment.

Survivin. Survivin is an apoptotic inhibitor not normally found in healthy adult tissue. It is selectively expressed during normal embryonic and fetal development, but is suppressed in adult cells. The role of survivin is to control apoptosis by manipulating mitotic progression and gene expression.[45] Tumor cells exploit and over-express this protein. Studies on survivin have found its presence in 78% of patients with bladder cancer, but not in normal patients. Reported sensitivity was 64%-100% and specificity was 87%-93%.[46] Preliminary studies with survivin have been lacking in appropriate validation criteria and data analysis. Further studies are needed to establish clinically relevant data.[47]

SUMMARY

The current protocols in place for bladder cancer screening are cystoscopy and urine cytology. Cytology does not have an adequate sensitivity in low-grade malignancy and has limited utility in the screening and management of bladder cancer patients. Urine tumor markers aimed at detection of cancer via voided urine are an attractive alternative to cytology. Currently, FDA-approved tumor marker assays lack the characteristics of an ideal test and have yet to revolutionize bladder cancer detection. Novel tumor markers, not yet FDA-approved, have the potential to change disease management algorithms that currently include voided urine cytology. Telomerase, an enzyme present in greater than 80% of all cancer cells, has the potential to be a successful bladder tumor marker for cancer surveillance and monitoring.

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REFERENCES

1. American Cancer Society: Cancer Facts and Figures 2008. Available from http://www.cancer.org/docroot/STT/STT_0.asp. Accessed 2008 April 1.
2. Carmack AJ, Soloway MS. The diagnosis and staging of bladder cancer: from RBCs to TURs. Urol 2006;67 (suppl 3A):3-5.
3. Sanchini MA, Gunelli, R, Nanni O, and others. Relevance of urine telomerase in the diagnosis of bladder cancer. JAMA 2005;294:2052-6.
4. Lokeshwar VB, Selzer MG. Urinary bladder tumor markers. Urol Oncol 2006 Nov-Dec;24(6):528-37.
5. TRAPeze XL Telomerase Detection Kit [package insert]. Temecula, CA: Chemicon International; 2006.

6. Sarosdy M, Hudson M, Ellis W, and others. Improved detection of recurrent bladder cancer using the Bard BTA-Stat test. Urol 1997;50:349-53.
7. Lokeshwar V, Soloway M. Current bladder tumor tests: does their projected utility fulfil clinical necessity? J Urol 2001;165:1067-77.
8. Raitanen MP, Hellstrom P, Marttila T, and others. Effect of intravesical instillations on the human complement factor h related protein (bta stat) test. Eur Urol 2001;40:422-6.
9. Thomas L, Leyh H, Marberger M, and others. Multicenter trial of the quantitative bta trak assay in the detection of bladder cancer. Clin Chem 1999;45:472-7.
10. Mahnert B, Tauber S, Kriegmair M, and others. Bta-trak a useful diagnostic tool in urinary bladder cancer? Anticancer Res 1999;19:2615-9.
11. Irani J, Desgrandchamps F, Millet C, and others. Bta stat and bta trak: a comparative evaluation of urine testing for the diagnosis of transitional cellcarcinoma of the bladder. Eur Urol 1999;35:89-92.
12. Heicappell R, Wettig IC, Schostak M, and others. Quantitative detection of human complement factor h-related protein in transitional cell carcinoma of the urinary bladder. Eur Urol 1999;35:81-7.
13. Shelfo SW, Soloway MS. The role of nuclear matrix protein 22 in the detection of persistent or recurrent transitional-cell cancer of the bladder. World J Urol 1997;15:107-11.
14. Miyanaga N, Akaza H, Tsukamoto T, and others Urinary nuclear matrix protein 22 as a new marker for the screening of urothelial cancer in patients with microscopic hematuria. Int J Urol 1999;6:189-214.
15. Ponsky LE, Sharma S, Pandrangi L, and others. Screening and monitoring for bladder cancer: refining the use of nmp22. J Urol 2001;166:75-8.
16. Yokoyama T, Sekigawa R, Hayashi T, and others. The clinical efficacy of BladderChek NMP22 in urothelial cancer. Rinsho Byori 2004;52:199-203.
17. Bergeron A, Champetier S, LaRue H. Maub is a new mucin antigen associated with bladder cancer. J Biol Chem 1996;271:6933-9.
18. Olsson H, Zackrisson B. Immunocyst a useful method in the follow-up protocol for patients with urinary bladder carcinoma. Scand J Urol Nephrol 2001;35:280-2.
19. Mian C, Pycha A, Wiener H, and others. Immunocyst: a new tool for detecting transitional cell cancer of the urinary tract. J Urol 1999;161:1486-9.
20. Vriesema JLJ, Atsma F, Kiemeney LALM, and others. Diagnostic efficacy of the Immunocyst test to detect superficial bladder cancer reoccurance. Urol 2001;58:367-71.
21. Blackburn EH. Telomeres and their synthesis. Science. 1990;249:489-90.
22. Greider CW, Blackburn EH. Telomeres, telomerase, and cancer. Sci Am 1996 Feb;274 (2):92-7.
23. Shea-Herbert B, Pongracz K, Shay J, and Grayaznov S. Oligonucleotide N3'-P5' phosphoramidates as efficient telomerase inhibitors. Oncogene 2002; 21: 638-43.
24. Harley CB, Futcher AB, Greider CW. Telomeres shorten during aging of human fibrobasts. Nature 1990;345:458-60.
25. Shay JW, Wright WE. Telomerase activity in human cancer. Current Opinion in Oncology 1996;8:66-71.
26. Shay JW, Wright WE. Telomerase: A target for cancer therapeutics. Cancer Cell 2002;2:257-65.
27. Lokeshwar VB, Soloway MS. Current bladder tumor tests: does their projected utility fulfill clinical necessity? J Urol 2001;165:1067-77.
28. Blackburn EH, Gall JG. A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena. J Mol Biol 1978;120:33-53.
29. Counter CM, Avilion AA, LeFeuvre CE, and others. Telomere shortening associated with chromosomal instability is arrested in immortal cells which express telomerase activity. EMBO J 1992;11:1921-9.
30. Kim NW, Piatyszek MA, Prowse KR, and others. Specifically association of human telomerase activity with immortal cells and cancer. Science 1994;266:2011-5.
31. Arai Y, Yajima T, Yagihashi A, and others. Limitations of urinary telomerase activity measurements in urothelial cancer. Clin Chim Acta 2000;296:35-44.
32. Konety BR, Nguyen TS, Brenes G, and others. Clinical usefulness of the novel marker BLCA-4 for the detection of bladder cancer. J Urol 2000;164:634-9.
33. Van Le TS, Miller R, Barder T, and others. Highly specific urine-based marker of bladder cancer. Urol 2005;66:1256-60.
34. Knudson W. Tumor-associated hyaluronan. Providing an extra-cellular matrix that facilitates invasion. Am J Pathol 1996;148:1721-6.
35. Lokeshwar VB, Obek C, Pham HT, and others. Urinary hyaluronic acid and hyaluronidase: markers for bladder cancer detection and evaluation of grade. J Urol 2000;163:348-56.
36. Lokeshwar V, Hautmann S, Schroeder G. Ha-haase test: an accurate test for monitoring bladder cancer recurrence and follow-up. J Urol 2000;163:132.
37. Sarkis AS, Charytonowicz E, Cardon-Cardo C, Fradet Y. Blood group antigen expression in bladder tumors: an immunohistochemical study of superficial bladder lesions. J Exp Clin Cancer Res 1994;13:139.
38. Pode D, Golijanin D, Sherman Y, and others. Immunostaining of Lewis X in cells from voided urine, cytopathology and ultrasound for noninvasive detection of bladder tumors. J Urol 1998;159:389-92.
39. Golijanin D, Sherman Y, Shpario A, Pode D. Detectino of bladder tumors by immunostaining of the Lewis X antigen in cells from voided urine. Urol 1995;46:173-7.
40. Van Rhijn BW, Lurkin I, Kirkels WJ, and others. Microsatellite analysis - DNA test in urine competes with cystoscopy in follow-up of superficial bladder carcinoma: a phase II trial. Cancer 2001;02;768-75.
41. Seripa D, Parrella P, Gallucci M, and others. Sensitivie detection of transitional cell carcinoma of the bladder by microsatellite analysis of cells exfoliated in urine. Int J Cancer 2001;95:364-9.
42. Witjes JA, van der Poel HG, van Balden MR, and others. Urinary nmp22 and karyometry in the diagnosis and follow-up of patients with superficial bladder cancer. Eur Urol 1998;33:387-91.
43. Van der Poel HG, van Balken MR, Schamhart DH, and others. Bladder wash cytology, quantitative cytology, and the qualitative bta test in patients with superficial bladder cancer. Urol 1998;51:44-50.
44. Van der Poel HG, van Rhijn BWG, Peelen P, and others. Consecutive quantitative cytology in bladder cancer. Urol 2000;56:584-8.
45. Altieri DC. Survivin, versatile modulation of cell division and apoptosis in cancer. Oncogene 2003;22:8481-9.
46. Shariat SF, Casella R, Khoddami SM, and others. Urine detection of survivin is a sensitive marker for the noninvasive diagnosis of bladder cancer. J Urol 2004;171:626-30.
47. Karam JA, Lotan Y, Shariat SF. Bladder cancer screening and future directions in urine-based markers for bladder urothelial carcinoma. Lab Med 2007;38:116-20.